BARD

3

FINAL REPORT

PROJECT NO. IS-1644-89C

מס' תיק: 1644-89C
תאריך: 1995

Effect of Dietary Iron on Lipid Peroxidation of Muscle Food

J. Kanner, D.D. Miller, I. Bartov, S. Harel

1995

630.72
BAR/KAN

2nd copy

44219

Appendix VI. BARD Cover Page for Scientific Reports

To be sent to BARD Office in Israel:
BARD, P.O. Box 6, Bet Dagan 50250, Israel.

Date:

BARD Project No. IS 1644-89C

Title **Effect of Dietary Iron on Lipid Peroxidation of Muscle Food**

All Investigators' Names
(Principal listed first)

J. Kanner	ARO Israel
D.D. Miller	Cornell, USA
I. Bartov	ARO, Israel
S. Harel	ARO, Israel

Project's starting date: January 1991

Type of Report: 1st Annual _____ 2nd Annual _____ Final ✓

Signature
Principal Investigator

Signature
Institution's Authorizing Official

630.72
BAR/KAN
2nd cery

B. TABLE OF CONTENTS

	PAGE
C. ABSTRACT	3
D. OBJECTIVES	5
INTRODUCTION	6
MATERIALS AND METHODS	13
RESULTS AND DISCUSSION	34
SUMMARY	115
CONCLUSIONS	118
BIBLIOGRAPHY	120
DESCRIPTION OF COOPERATION	132
EVALUATION OF THE RESEARCH ACHIEVEMENTS	133
LIST OF PUBLICATIONS	136

THE EFFECT OF DIETARY IRON LEVEL ON LIPID PEROXIDATION OF MUSCLE FOOD

C. ABSTRACT

Biological oxidations are almost exclusively metal ion-promoted reactions and in this respect iron, being the most abundant, is the commonly involved. The effect of dietary iron levels on pork, turkey and chick muscle lipid peroxidation and various other related compounds were evaluated. Crossbred feeder pigs were fed to market weight on corn-soy rations containing either 62,131 or 209 ppm iron. After slaughter, the muscles were dissected, cooked and stored at 4°C. Heavily fortifying swine rations with iron (>200 ppm) increase non-heme iron (NHI), thiobarbituric acid reactive substances (TBARS), and decrease α -tocopherol in cooked stored pork but did not increase warmed-over aroma (WOA). NHI and TBARS were higher in cooked pork from pigs fed high-iron diets. Liver iron correlated with muscle iron. TBARS were strongly related with WOA. The role of dietary vitamin E and ascorbic acid on Fe-induced *in vivo* lipid peroxidation in swine was also evaluated. Moderate elevation in iron stores had a marked effect on oxidative stress, especially as indicated by liver TBARS. Supplemental vitamin E, and to a lesser extent vitamin C, protect against this oxidative stress. Unsupplementation of Fe in the regular diet of turkeys did not affect body weight, blood hemoglobin level, or iron pool in the liver or muscle. The reason being that it contained "natural" ~120 mg Fe/kg feed, and this amount is high enough to keep constant the pool of iron in the body, liver or muscle tissues. Only Fe-supplementation with high amounts of Fe (500 ppm)

significantly increased turkey blood hemoglobin and total iron in the liver, in 1 out of 3 experiments, but only slightly affects iron pool in the muscles. It seems that the liver accumulates very high concentrations of iron and significantly regulates iron concentration in skeletal muscles. For this reason, it was very difficult to decrease muscle stability in turkeys through a diet containing high levels of Fe-supplementation. It was shown that the significant increase in the amount of iron (total and "free") in the muscle by injections with Fe-dextran accelerated its lipid peroxidation rate and decreased its α -tocopherol concentration. The level and metabolism of iron in the muscles affects the intensity of *in vivo* lipid peroxidation. This process was found to influence the turnover and accumulation of α -tocopherol in turkey and chick muscles. Treatments which could significantly decrease or increase the amount and metabolism of iron pool in muscle tissues (or other organs) may affect the rate of lipid peroxidation and the turnover of α -tocopherol. Several defense enzymes were determined and found in the turkey muscle, such as superoxide dismutase, catalase, and glutathione peroxidase. Glutathione peroxidase was more active in muscles with a high trend of lipid peroxidation, more so in drumsticks than in breast muscles, or muscles with a low α -tocopherol content. The activity of glutathione peroxidase increased several fold in muscle stored at 4°C. Our work demonstrated that it will be much more practical to increase the stability of muscle tissues in swine, turkeys and chickens during storage and processing by increasing the amount of vitamin E in the diet than by withdrawing iron supplementation.

D. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

- a.** To evaluate to what extent the iron supplement of poultry/pigs can be reduced without interfering in their growth, feed efficiency and health.
- b.** To evaluate the effect of avian/animal dietary iron level/low, medium and high) on after slaughtering muscle lipid peroxidation.
- c.** To determine the link between iron supplementation and its accumulation and distribution in muscle tissues.
- d.** To study the effect of dietary iron on the activity of several catalyzers of lipid peroxidation (free iron, lipoxygenase, membranal enzymic system) and the antioxidant system (α -tocopherol, SOD, catalase, glutathione-peroxidase) in muscle tissues.
- e.** To study the effect of dietary Fe level on muscle lipid peroxidation, during processing and storage of muscle foods, with and without precooking, stored at 4°C and -18°C).

Due to budget changes by BARD administration, the research focus was directed in particular on objectives (a) and (b), and only partially on objectives (c), (d) and (e).

INTRODUCTION

Lipid peroxidation is one of the primary mechanisms of quality deterioration in stored foods of animal origin. The changes in food quality are manifested by a deterioration in flavor, color, texture, nutritive value and the production of toxic compounds (Simic and Karel, 1980; Khayat and Schwall, 1983; Ames, 1983; Addis, 1991; St. Angelo and Bailey, 1987).

Phospholipids play a major role in the develop of off-flavor in turkey, chicken, beef and lamb, but that total lipids are the major contributor to off-flavors in pork (Wilson *et al.*, 1976; Igene and Pearson, 1979) verified that phospholipids were the major contributors to off-flavors. The ability of phospholipids to oxidation is at least partially due to their high content of PUFA, particularly arachidonic acid.

Processes which disrupt the membrane function expose it to oxygen, metal ions and enzymes, cause a rapid development of rancidity in fresh and cooked muscles.

Minced muscle tissues generate hydrogen peroxide (Harel and Kanner, 1985) which activates oxy- or metmyoglobin to ferryl compound (Kanner *et al.*, 1986). Ferrylmyoglobin oxidizes membrane fatty acids (Kanner and Harel, 1985a), and also other molecules, especially reducing compounds (Kanner and Harel, 1985b). Hydrogen peroxide is only one of the cytosolic compounds which can affect lipid peroxidation in muscle foods.

Reducing compounds are among the other important compounds which may affect the process of lipid peroxidation. H₂O₂-activated metmyoglobin, in the presence of the cytosolic fraction which contains high concentration of reducing agents and catalase, is not an initiator of muscle lipid peroxidation (Harel and Kanner, 1989; Kanner *et al.*, 1991b).

Data indicated that initiation of lipid peroxidation in injured biological tissues and muscle foods are generated by "free" iron ions and hydrogen peroxide produced at a site-specific (Halliwell and Gutteridge, 1985; Kanner *et al.*, 1986, 1988a, 1988b; Aust, 1988) via the following mechanism:

or

This reaction is cycled by a superoxide (Haber-Weiss) produced enzymatically or non-enzymatically, or by a reducing compound, e.g. ascorbic acid (redox cycle) (Kanner *et al.*, 1987). Furthermore, ferrous ions especially work to propagate lipid peroxidation by breakdown hydroperoxides to free radicals.

The initiation of lipid peroxidation in raw muscle tissues is mostly affected by free iron ions which are redox-cycled by non-enzymic or enzymic reactions. Several studies have shown that lipoxygenase may participate in the process of muscle lipid peroxidation (German and Kinsella, 1985; Grossman *et al.*, 1988; Hsieh and Kinsella, 1989).

Muscle lipid peroxidation in precooked products seems to only derive from a non-enzymic reaction dependent on "free" metal ions. Heme proteins seem to affect lipid peroxidation in muscle foods only after continuous heating, which decreases the amounts of the reducing compounds to a low level. At this stage, heme proteins especially affect the propagation process by breaking down hydroperoxides to free radicals (Kanner, 1974).

Biological oxidations are almost exclusively metal ion-promoted reactions and in this respect iron, being the most abundant, is the commonly involved.

In mammalia, about two-thirds of iron is present in hemoglobin. A further 10% is found in myoglobin - a very small amount as prosthetic components in various iron-containing enzymes, and in the transport plasma protein transferrin. The remainder is present in intracellular storage proteins, ferritin and hemosiderin. A small pool of nonprotein-bound iron equilibrating between transferrin, cell cytoplasm, mitochondria and ferritin provide "free" iron at micromolar concentrations in tissues (Jacobs, 1977; Aisen and Liskowsky, 1980). It was found by us that the amount of "free" iron in muscle turkey is of 0.5-2.0 $\mu\text{g/g}$ f.wt (Kanner *et al.*, 1986).

The majority of intracellular iron is deposited in ferritin. Ferritin can contain up to 4,500 ferric ions per ferritin complex. Iron is mobilized from ferritin by reduction, but the physiological reductant remains unknown. Chelating agents are required, along with reductant, but again the physiological chelator is unknown. It is now hypothesized that iron release

from ferritin by O_2 is the source of iron for the O_2^- and H_2O_2 -dependent (Haber-Weiss) reaction, which generate lipid peroxidation and other cytotoxic effects to biological systems (Biemond *et al.*, 1986; Boyer and McCleary, 1987).

Another major pool of iron in muscle cells is myoglobin. Gutteridge (1986) and others (Chen *et al.*, 1984; Rhee *et al.*, 1987; Harel *et al.*, 1988) have shown that iron can be released from myoglobin or hemoglobin by H_2O_2 . Our results show that in the presence of physiological amounts of H_2O_2 and reducing agents, the release of iron from hemoproteins is very low (Harel *et al.*, 1988). However, it may be possible that heating could induce the release of iron from myoglobin at significant amounts (Chen *et al.*, 1984). It seems that the low molecular weight "iron pool" or the "free" iron may, in fact, be the most important pools of redox active iron available to catalyze the destruction oxidation of biomolecules in biological systems (Biemond *et al.*, 1986; Halliwell and Gutteridge, 1990).

Recently, forms of non-heme iron such as ferritin, cytosolic iron-dependent enzymes, and low molecular weight (LMW) chelatable iron ions have been shown to enhance lipid peroxidation in meat (Decker and Hultin, 1990; Decker and Welch, 1990; Graf and Panter, 1991; Kanner and Doll, 1991; Kanner *et al.*, 1991a; Kanner *et al.*, 1991b; Kanner *et al.*, 1991c; Seman *et al.*, 1991) and *in vivo* systems (Halliwell and Gutteridge, 1986). Human and animal studies have linked high iron stores with *in vivo* lipid peroxidation (Dougherty *et al.*, 1981; Kanner *et al.*, 1990; Lee *et al.*, 1981), LDL oxidation (Steinberg *et al.*, 1989), and atherogenesis (Murray *et al.*, 1991; Salonen *et al.*, 1992).

Chickens, turkeys and swine exposed to iron deficiency tend to develop anemia, characterized by a decrease in the concentration of blood hemoglobin and packed cell volume, as well as in growth retardation. Based on these parameters, the iron requirements for broilers are 80 mg/kg diet, and for young and grown turkeys 80 and 50 mg/kg diet, respectively. The iron requirements of the pigs are for young animals (1-10 kg) (10-20 kg), and for mature (20-50 kg) (50-110 kg), 100, 80, 60 and 40 mg Fe/kg diet, respectively (N.R.C., 1988). However, the levels currently in use by many hog producers in the U.S. are 250 mg Fe/kg diet.

Iron overload toxicity is a major life-limiting complication of several diseases managed by continued blood transfusions in humans (for review see Jancu, 1982; Herskho and Weatherall, 1988). Evidence for increased *in vivo* lipid peroxidation has been produced in humans iron-overload (Heys and Dormandy, 1981; Rachmilewitz *et al.*, 1976), as well as in the experimental animals (Dougherty *et al.*, 1981; Dillard *et al.*, 1984; Dabbagh *et al.*, 1994; Marklund *et al.*, 1983; Nayini *et al.*, 1985).

In recent years, iron and dependent oxygen-active species have been implicated in the pathogenesis of several human diseases, including atherosclerosis, auto-immune and inflammatory joint diseases, cardiac and other tissue ischemia, aging and cancers (for review see Halliwell and Gutteridge, 1990). The effect of iron-overload on the quality of muscle products was not studied in the past.

Control of lipid peroxidation. Available methods for controlling lipid peroxidation may be divided into two categories, i.e. those acting to

control the level of oxygen and active oxygen species, and those acting to control activated catalysts and free radicals.

Control of oxygen and oxygen species. The level of available oxygen may be controlled in foods by specific processing, such as de-aeration or vacuum-packaging. It may also be controlled by oxygen scavengers, such as a glucose oxidase/catalase system which reduces oxygen to H₂O.

The main protective mechanisms used by cells *in vivo* against the toxic effects of oxygen are enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Chan and Decker, 1994; Fridovich, 1983; Marklund, 1985).

Control of active catalysts and free radicals. Iron is mostly kept tightly bound to functional proteins, however a small pool is in transit between storage proteins (ferritin), transport proteins (transferrin), and functional proteins. Chelating agents can significantly affect the kinetics of lipid peroxidation induced by metals (Anon, 1988). Numerous studies with EDTA have demonstrated the complexity which it imparts upon the reactivity of iron. This was summarized by us (Kanner *et al.*, 1986; Kanner *et al.*, 1987). Polyphosphates are well-known chelators of iron, which could control lipid peroxidation in minced, cooked meat (Anon, 1988; Kanner, 1994).

For convenience, antioxidants are divided into two classes. Preventative, or primary, antioxidants reduce the rate of initiation of free radical chains, usually by converting the free radical producing hydroperoxidic products (ROOH) to innocuous products (such as alcohols) (Kanner *et al.*, 1987).

Chain-breaking, or secondary, antioxidants trap the chain-propagation radicals. The majority of chain-breaking antioxidants are phenols. The major synthetic antioxidants in common foods used are BHA (butylhydroxyanisole), BHT (butylhydroxytoluene), TBHQ (t-butylhydroxyquinone), propyl gallate, and ascorbyl palmitate. Vitamin E (α -tocopherol) is a natural fat-soluble molecule that concentrates in the hydrophobic interior of biological membranes (Dillard *et al.*, 1984). Tocopherols are effective antioxidants in many foods; they have been tested and found effective in bacon, baked goods, butterfat, lard, and numerous other foods (Dugan, 1980). Stability of carcass fat and meat of broiler chicks and turkeys towards oxidation is markedly dependent on the amount of dietary vitamin E and the type of synthetic antioxidant used. Increasing the amount of dietary vitamin E and using the synthetic antioxidant, ethoxyquin, but not BHT and Endox[®], improves meat and fat stability (Bartov and Burnstein, 1977). Most recently, supplementation of dietary vitamin E was found to increase the stability of myoglobin and lipids toward peroxidation in displayed beef (Arnold *et al.*, 1993). Synthetic antioxidants, such as BHA, BHT and TBHQ, and natural-occurring substances, may be used successfully to prevent lipid peroxidation in muscle tissue, but only in minced products (Kanner *et al.*, 1984; Kanner *et al.*, 1988b). Lipid peroxidation could be inhibited very effectively in cured meat by nitrite, which generates NO $\cdot$. Nitric oxide was found by us and others to inactivate iron catalysts as promoters of lipid peroxidation (Anon, 1988a; Kanner *et al.*, 1984). Our previous studies show that "free" ions are the most important.

Our previous studies show that "free" (chelatable) iron ions are the most important catalysts of *in situ* muscle lipid peroxidation (Kanner *et al.*,

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
B	Breast
BHA	Buthylhydroxyanisole
BHT	Buthylhydroxytoluene
BOOH	Buthylhydroperoxide
BW	Body weight
Ca BP	Calcium binding protein
CD	Commercial diet
CHOL	Cholecalciferol
D	Drumstick
DFA	Deferrioxamine
DTPA	Diethylenetriamine pentaacetic acid
EDTA	Ethylenediaminetetra-acetic acid
FAME	Fatty acid methyl esters
f. wt.	Fresh weight
FX	Ferrioxamine
GMs	Grand means
GSH	Reduced glutathione
GSHPx	Glutathione peroxidase
GSR	Glutathione reductase
GR	Grounds pork
Hb	Hemoglobin
1-HYD	25(OH)D ₃ - 1 - hydrolase
24-HYD	25(OH)D ₃ - hydrolase
IGM	Individual grand mean
L	Left drumstick
LD	Longissimus dorsi
MDA	Malondialdehyde
NADPH	Nicotinamide adenin dinucleotide phosphate, reduced form.
NHI	Non heme iron
PCV	Packed cell volume
PT	Plasma Tocopherol
PUFA	Poly unsaturated fatty acids
R	Right drumstick
RBC	Red blood cell
RF	Rectus femoris
SLP	Slopes
SI	Serum iron
SDS	Sodium dodecyl sulfate
SOD	Superoxide dismutase
TBARH	Thiobarbituric acid reactive substances
TBHQ	T-buthyl hydroxy quinone
TCA	Trichloroacetic acid
TEP	1,1,3,3 Tetraethoxypropane
TGM	Treatment grand means
TI	Total iron
TIBC	Total iron binding capacity
TS	Transferrin saturation
WISP	Waters intelligence sample processor
WOA	Warmed - over aroma
WOF	Warmed - over flavor

1988a, 1988b, 1988c). These results led us to develop a preliminary study in order to evaluate whether the stability of *in situ* turkey muscle to the process of lipid peroxidation could be improved when Fe supplement is omitted from the diet feed during the last growth period (Kanner and Bartov, 1988; Kanner *et al.*, 1990).

The present study included turkeys, chicken and swine in a research to evaluate the effect of dietary iron level on muscle lipid peroxidation.

MATERIALS AND METHODS

Birds (Turkey) and Diets

Day-old males of a large strain of Broad Breasted White turkeys (British United Turkeys, T6) were raised in electrically heated battery brooders placed in a temperature-controlled room at $25^{\circ}\pm 2^{\circ}\text{C}$ up to 28 d of age, and then in individual cages with wire floors in a temperature-controlled ($22^{\circ}\pm 2^{\circ}\text{C}$) building. Twenty-four hours of light/day were provided in both rooms. After a preparatory period of 7 d, two-thirds of the birds (those closest to the average weight) were wing-banded and divided into groups of 11 birds each, according to body weight. Both mean group weights and individual weight distribution were equal within the groups. Two such groups were assigned to each of the experimental diets (see below). The basal diets (Table 1) were formulated for the age periods 0-4, 4-8, 8-12, 12-16, 16-20 and 20-24 weeks, according to the National Research Council (NCR, 1984). The mineral mix used in experiment 1 did not contain Fe supplement, otherwise it provided 20 mg Fe/kg diet. The diets fed in experiments 2 to 5, since the age of about 8 weeks, contained

vitamin mix from which DL α -tocopheryl acetate (vitamin E) was omitted and ethoxyquin was replaced by the same concentration of BHT. The basal diets were supplemented with various levels of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Fluka Chemie A.G., CH-7470 Buchs, Switzerland), vitamin E (ROVIMIX E-50 SD; Hoffmann-La Roche, CH-4002 Basel, Switzerland), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Baker Chemical Co., Phillipsburg NJ, USA) (see below) and were fed *ad libitum* in mash form.

Management (Turkey)

Individual body weights were recorded bi-weekly. Food consumption data were obtained on a group basis. The number of birds per treatment was gradually reduced to five by the termination of the experiments, either due to slaughtering for the evaluation of meat stability during the course of the study (Experiments 1 and 2), or by eliminating birds with health problems (leg disorders, enlarged crops, underweights). Birds selected for slaughtering were those whose body weights were within $\pm 7.5\%$ of the treatment average. Blood was taken from the brachial vein using a heparinized syringe, during the course of the experiments and at their termination, to determine hemoglobin (all experiments), and calcium (Ca) and phosphorous (P) (Experiment 2). At slaughtering, either during the course of the experiments or at their termination at 22-23 weeks of age, meat from the thigh and the drumstick (the muscle around the femur and the tibia, respectively) was removed and stored at -18°C and 3°C , respectively, for various periods to determine its stability. After sampling, it was returned to -18°C . Livers were also removed at slaughtering and kept at -18°C for further tests.

Birds (Chicks) and Diets

Day-old White Rock (Cobb) male chicks were weighed, wing-banded and divided, according to body weight, into 4, 3, 7 and 5 groups of 10 chicks each in experiments 1, 2, 3 and 4, respectively. Both mean group weights and individual weight distribution were equal within the groups. Each group was allotted to one of the experimental diets, based on Fe-deficient synthetic starter and grower diets (Table 2), whose nutrient content was based on the recommendations of the NRC (1984). These diets, supplemented with various levels of cholecalciferol (vitamin D₃, NBCo Nutritional Biochemicals Corporation, Cleveland, Ohio, 44128, U.S.A.) and Fe (FeSO₄·7H₂O, Fluka Chemie A.G., CH-9470 Buchs, Switzerland) were fed *ad libitum* - in mash form - from the first day of life until the termination of the experiments. In each experiment, one group was fed on a commercial corn-soybean diet as a control. The chicks were raised in groups up to 4 weeks of age in electrically-heated battery brooders, placed in a temperature-controlled room at 25°C, and thereafter in individual cages placed in a temperature-controlled room at 20°C. Continuous light was provided in both rooms.

Management (Chicks)

Individual body weights and feed consumption data, on a group basis, were obtained at intervals of 6 to 7 days. At the termination of the experiments, the chicks were bled by cardiac puncture using a heparinized syringe to determine hemoglobin, calcium and phosphorous. After slaughtering, the drumsticks (the muscle around the tibia) were separated and stored at 3°C to determine the meat stability. The livers were removed and weighed. The kidneys and the duodenum were also

separated and stored at 3°C to determine the meat stability. The livers were removed and weighed. The kidneys and the duodenum were also removed and placed in ice-cold sterile saline to measure the cholecalciferol hydroxylase activity and the calbindin content, respectively. The tibiae were removed to determine ash content.

Swine Feeding Study

Animals and Diets. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Cornell University.

Fifteen crossbred barrows were selected on a similar weight basis (24.7 ± 1.3 kg) and allocated to one of three groups, so that each group had similar mean body weights. Groups were then randomly assigned to the three treatment diets (Table 3). Prior to the study, swine were injected with 100 mg iron dextran at 3 days of age, weaned at 21 days of age, and fed commercial creep pellets (Vigortone® VigorStart NT Pig Starter Medicted, Vigortone® Ag Products Inc., Cedar Rapids, IA, USA) containing 580 ppm iron (by analysis) up to 25 kg mean body weight.

Corn-soy rations were formulated to contain 16% protein for the first 6 weeks of the study (59 kg mean body weight) and 14% protein for the remaining 7 weeks, thus satisfying the NRC (1988) requirements (Table 3). Mean dietary iron level for each treatment diet was calculated based on the average iron content in the 16% and 14% protein rations. These values were used in the statistical analyses. The high-iron diet was formulated using ingredients and levels commonly fed by U.S. swine

achieved by using iron-free vitamin/mineral mix, as well as reagent grade calcium phosphate in place of feed grade calcium phosphate which carries significant quantities of iron. By formulation, all diets had a Ca:P ratio of 0.55:0.44 and contained 30 IU vitamin E/kg. Due to time constraints, diets were analyzed for vitamin E several months after the feeding study. Therefore, the actual α -tocopherol values (Table 3) may be lower than α -tocopherol content as fed.

Housing and Feeding. Animals were individually housed at or above 70°F in a temperature-regulated building on unbedded concrete floors in 8 x 3 ft epoxy coated (IronClad®, Benjamin Moore & Co., Montvale, NJ, U.S.A.) galvanized metal pens equipped with automatic stainless steel nipple water units and 30 kg-capacity wooden feeders. A 5-micron water filter (W.W. Grainger, Inc., Catalog #377, Cartridge #110, 9¾ x 2¾ inch diameter cartridge for 14 5/8 inch pump filter) held iron contamination in the water to less than 2 ppm by analysis. Feed and water were provided *ad libitum*, and pens were rinsed daily. Weekly feed intakes, rates of gain, and feed efficiencies were calculated throughout the 13-week study.

TABLE 1. Composition of the Basal Diets (Turkeys)

Ingredients	Age period (weeks)					
	0-4	4-8	8-12	12-16	16-20	20-24
	%					
Yellow corn	20.00	25.00	25.00	25.00	25.00	25.00
Sorghum grains	17.55	15.22	27.18	35.8	41.35	49.15
Soybean meal (44.5% protein)	52.30	51.00	39.00	30.40	24.40	16.60
Fish meal	2.00	-	-	-	-	-
Acidulated soybean soapstock	3.40	5.00	5.00	5.00	5.50	5.50
DL-Methionine	0.2	0.13	0.12	0.10	0.05	0.05
L-Lysine	-	-	0.50	0.05	0.05	0.05
Dicalcium phosphate	2.10	2.00	2.00	2.00	2.00	2.00
Limestone	1.80	1.00	1.00	1.00	1.00	1.00
Mineral mix ¹	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ²	0.25	0.25	0.25	0.25	0.25	0.25
Calculated values						
AME _n (kcal/kg)	2794	2945	3068	3157	3250	3330
Crude protein	28.10	26.40	22.20	19.10	16.90	14.20
Crude fat	4.90	6.20	6.50	6.70	7.20	7.40
Calcium	1.40	0.99	0.96	0.93	0.92	0.90
Phosphorous	0.86	0.79	0.74	0.71	0.69	0.66

¹Supplying (in mg/kg feed): sodium chloride, 2500; sodium sulfate, 1000; Mn, 80; Zn, 75; Fe, 20; Cu, 5; I, 1.2; Co, 0.2; Se, 0.3.

²Supplying (per kg of food): retinyl acetate, 3.44 mg; cholecalciferol, 72.5 µg; DL α -tocopherol acetate, 28 mg; menadione sodium bisulphite, 2 mg; riboflavin, 7.2 mg; calcium pantothenate, 12.8 mg; niacin, 64 mg; cyanocobalamine, 0.012 mg; folacin, 1.2 mg; pyridoxine hydrochloride, 3.6 mg; thiamine hydrochloride, 1.2 mg; biotin, 0.12 mg; choline chloride, 250 mg; ethoxyquin, 62.5 mg; BHT, 62.5 mg.

TABLE 2. Composition of the Basal Synthetic Fe-Deficient Diets (%) for Chicks

Ingredients	Age period (weeks)	
	0-4	4-7
Glucose monohydrate	58.22	63.54
Casein	28.00	23.00
L-Arginine	1.20	1.00
DL-Methionine	0.22	0.10
Soybean oil	4.00	4.00
Cellulose (Solka floc)	3.00	3.00
Mineral mix ¹	4.81	4.81
Choline chloride (50%)	0.30	0.30
Vitamin mix ²	0.25	0.25
Calculated values		
AME _n (kcal/kg)	3437	3408
Crude protein	25.84	21.16
Energy-to-protein ratio	133.30	161.00
Lysine	2.24	1.84
Arginine	2.21	1.83
Sulfur amino acids	1.01	0.75
Crude fat	4.22	4.18
Calcium	1.08	1.05
Phosphorous	0.63	0.58

¹Supplying (per kg of feed): CaCO₃, 17g; CaHPO₄·2H₂O, 10 g; KH₂PO₄, 7.5 g; NaHCO₃, 3.2 g; KHCO₃, 3 g; MgCO₃, 2.51 g; KCl, 2 g; NaCl, 1.6 g; Na₂SO₄, 1 g; MnSO₄·H₂O, 0.201 g; ZnO, 54 mg; CuSO₄·5H₂O, 34.2 mg; KI, 1.7 mg; CoCl₂·6H₂O, 0.8882 mg; Na₂MoO₄·2H₂O, 0.5548 mg; Na₂SeO₃, 0.482 mg. This mix supplied (per kg feed): K, 4.34 g; Na, 1.63 g; Cl, 1.63 mg; Mg, 0.652 g; Mn, 65.2 mg; Zn, 43.4 mg; Cu, 8.69 mg; I, 1.3 mg; Se, 0.22 mg; Co, 0.22 mg; Mo, 0.22 mg.

²Supplying (per kg of feed): retinyl acetate, 3.096 mg; cholecalciferol, 37.5 µg; DL α-tocopheryl acetate, 24 mg; menadione sodium bisulphite, 2 mg; riboflavin, 12 mg; calcium pantothenate, 30 mg; niacin, 100 mg; cynocobalmine, 0.03 mg; folacin, 2 mg; pyridoxine hydrochloride, 10 mg; thiamine hydrochloride, 6 mg; biotin, 0.5 mg; BHT, 125 mg.

TABLE 3. Composition of treatment diets (swine)

Ingredient (%) ^a	Low Fe		Medium Fe		High Fe	
	Protein Level					
	16%	14%	16%	14%	16%	14%
Ground Corn ^b	76.39	82.12	76.04	81.92	76.04	81.92
Soybean Meal ^c	21.25	15.88	21.25	15.88	21.25	15.88
Ca Phosphate ^d	0.00	0.00	0.96	0.54	0.96	0.54
RG CaHPO ₄ ^e	0.89	0.48	0.00	0.00	0.00	0.00
Ca Carbonate ^f	0.87	0.92	1.15	1.06	1.15	1.06
Salt ^g	0.35	0.35	0.35	0.35	0.35	0.35
+Fe Vit/Min. mix ^h	0.00	0.00	0.00	0.00	0.25	0.25
-Fe Vit/Min. mix ⁱ	0.25	0.25	0.25	0.25	0.00	0.00
Nutrient ^j						
Iron (ppm)	68.9	55.7	148.8	113.0	226.6	191.1
Mean Iron (ppm)	62.3		130.9		208.8	
α-toco (IU/kg)	24.5	25.1	22.9	24.1	19.5	20.4
Mean α-toco (IU/kg)	24.8		23.5		20.0	

^aBy formulation.

^bShelled yellow corn; 8.2% protein; 0.03% Ca; 0.28% P; 0.003% Fe; 7.98 mg/kg α-tocopherol.

^cDehulled soy meal; 48% protein; 0.26% Ca; 0.64% P; 0.016% Fe; 2.03 mg/kg α-tocopherol.

^dNutrena® (Cargill, Minneapolis, MN) Dicalcium/Monocalcium Phosphate; ≥15% Ca; ≥20.5% P; 1.29% Fe.

^eReagent Grade Dibasic Calcium Phosphate (Sigma, #C-7263, St. Louis, MO); 29.46% Ca; 22.77% P; 0.0135% Fe.

^fGranular CaCO₃ (Limestone Products Corp., Sparta, NJ); (Limestone Products Corp., Sparta, NJ); ≥38% Ca; 0.0144% Fe.

Hematology. Blood was drawn biweekly from the anterior vena cava using both 3 ml heparinized and 7 ml non-heparinized tubes (Vacutainer™, Becton Dickinson Co., Rutherford, NY). Swine were partially restrained by a snout snare during collection.

Serum was separated by 15-minute centrifugation at 850-1000 RCF (2400 rpm) (CRU-5000 International Equipment Co., Needham Hts., MA). Samples were refrigerated and analyzed in duplicate within 24 hours for

serum iron (SI) and total iron-binding capacity (TIBC) (Sigma Chemical Co., St. Louis, MO, Kit #565-B).

Hemoglobin (Hb) (Sigma Chemical Co., St. Louis, MO, Kit #525-A) and packed cell volume (PCV) determinations were made in triplicate using heparinized blood.

Plasma tocopherol (PT) was determined at slaughter by the method of McShane *et al.* (1991).

Blood hemoglobin concentration in turkey and chicks were determined according to Anon (1984).

Slaughter and ground pork preparation. Slaughtering took place on three separate days within the 13th week of the study. On a given slaughter day, five pigs were fasted overnight and killed by humane electrical stunning and exsanguination. Slaughter order was based on body weight and treatment, starting with the heaviest animal within each treatment. Venous blood was collected at slaughter for SI, TIBC, Hb, PCV and PT determinations. Carcasses were eviscerated, split, and chilled at 4°C overnight. Boar odor may occur in cooked pork from boars as well as barrows (Pearson and Tauber, 1984).

Tenth rib backfat and loin eye were measured in each carcass half. Fifteen pounds of GP were prepared from deboned, 1/8 inch fat-trimmed Boston Butt and Picnic cuts from the right carcass half. Pork from each animal was ground in two batches in a stainless steel bowl chopper (Hobart Manufacturing Co., Troy, OH) for 60 seconds. Both batches of

chopped pork were then blended in a paddle mixer for 60 seconds. Poorly ground pieces, high in connective tissue, were discarded.

Packaging. Samples were vacuum-packaged (Multi-Vac vacuum package machine, type AG5, Sepp Haggemuller KG, W. Germany) in pouches (FreshPak 500™, Koch Supplies Inc., Kansas City, MO, Code #01-46-00-A) in portions of 25-30 g (lipid determinations), 50-75 g (all other chemical assays), or 100 g (sensory panel), and stored at either -70°C (samples for lipid analysis), or -40°C (remaining samples). The pouches were made from laminated nylon/polyethylene with the following specifications (supplied by the manufacturer): 6 x 8.5 inch o.d., 3 mils, O₂ transmission rate = 3.5 cc/100 sq. in/24 h at 70°F, water vapor transmission rate = 0.6 g/100 sq. in/24 h at 100°F.

Samples of turkey and chicks were packaged in polyethylene pouches and stored at -18°C and -70°C for analysis.

Cooking procedure

One day prior to cooking, pork was thawed overnight at 4°C, and pouches were examined for seal and vacuum imperfections. Poorly sealed or poorly evacuated bags were repackaged before cooking.

Pouches were flattened to 3/4 in. thicknesses, slit 1/2 in. above the vacuum seal (without breaking seal), slid onto 1/8 in. rods in a handmade cooking rack apparatus, and submerged in water held at 80°C in a steam-jacketed, 50-gallon stainless steel rectangular cheese vat. The vacuum seal on one extra bag of pork was punctured, and internal temperature

was monitored using a digital probe thermometer (Omega Engineering Inc., Stamford, CT, #701JY0186). Samples were removed from the bath 10 minutes after reaching an internal temperature of 76°C. Cooking time averaged 15 minutes per batch.

Cooking procedure of turkey thigh was done in nylon/polyethylene laminate pouches which were submerged in water held at 80°C and internal temperature was monitored using a thermometer. Samples were removed from the bath 10 minutes after reaching an internal temperature of 70°C.

Storage study

After cooking, each bag was opened and its contents transferred to and sealed in a pre-labeled pleated sandwich bag with zipper (Ziploc®, DowBrands, Indianapolis, IN, 6 1/2 x 5 7/8 in, poly-ethylene, 1.15 mil, O₂ transmission rate = 424 cc/100 sq.in/24 h at 73°F, water vapor transmission rate = 1.0 - 1.5 g/100 sq. in/24 h at 100°F and 90% relative humidity). Bags of cooked pork were stored in non-overlapping layers on trays at 4°C for 0-12 days. One bag from each pig was pulled on each of 5 storage days (day 0, 2, 4, 8, 12) for chemical analyses, and 3 bags for the same 5 days were pulled for sensory evaluation.

Fresh and cooked turkey thighs were transferred to polyethylene bags and sealed in the presence of air, and stored at 4°C for 0-10 days. Each day the bag was opened to air and then resealed.

Chemical analyses

TBARS test. The method of Witte *et al.* (1970), as modified by Willemot *et al.* (1985), was used to measure lipid oxidation in triplicate samples from each pig. The procedure was further modified by adding butylated hydroxytoluene (BHT) and EDTA prior to blending, to minimize sample oxidation during the assay (Pikul *et al.*, 1989; Kanner, 1991). Five g of crumbled, ground muscle was weighed into a 250-ml glass beaker and the following reagents were added: 1 ml BHT solution (1 mg/ml in ethanol) and 44 ml extracting solution (10% TCA in 0.1 M H_3PO_4). Each sample was blended directly in the beaker for 30 seconds on high speed, using an upright hand. The blender was rinsed with distilled water between samples. Recovery was measured in 3 spiked pork samples containing 12 ml 1,1,3,3-tetraethoxypropane (TEP) standard (10^{-5} M), 1 ml BHT, and 32 ml extracting solution. Sample blanks were carried through for each sample. TBARS were expressed as μg malonaldehyde (MDA)/g sample (wet weight).

Iron analysis. All glassware was acid-washed in 1 N HCl and rinsed with distilled water prior to use.

NHI concentration was measured by modifications of the methods of Schricker *et al.* (1982) and Rhee and Ziprin (1987). Two hundred μl of 0.93% NaNO_2 was added prior to incubation, to stabilize the heme complex and thereby minimize the release of heme iron during the 20 h incubation at 65°C . After heating, each sample was assayed as follows: 1 ml extract was combined with 1 ml hydroxylamine hydrochloride (3% w/v in distilled water, Sigma, H-2391), vortexed, and held 10 minutes. One and a half ml bathophenanthroline disulfonic acid (0.3 mg/ml in 3 M

sodium acetate, Sigma, B-1375) was added as the color reagent and, after 15 minutes absorbance was recorded at 533 nm. Sample blanks (3 M sodium acetate replaced the bathophenanthroline reagent) were carried through to correct for dark-colored extracts. A standard curve was prepared using an iron atomic absorption standard (Sigma, catalog #I-9011).

“Free” chelatable iron measurements

“Free” iron was determined by a method developed by Gower *et al.* (1989). The method uses desferrioxamine to chelate “free” iron from biological tissues.

Muscle tissues were blotted dry, weighed and homogenized to a 20% w/v homogenate with Chelex-100 treated distilled water, was centrifuged at 15,000 g for 15 min, and 1 ml were taken from the supernatant, brought to 2mM deferrioxamine (DFA) by adding 100 μ l of 22 mM DFA and was incubated at 37°C for 30 min. The incubations were stopped by passing the sample through a Sep-Pak C18 (Millipore Corp., Milford, MA), pre-eluted with ethanol, followed by 5 ml distilled water on which ferrioxamine and DFA are retained. After application of the sample, it was washed with 5 ml water and then 2 ml ethanol which eluted FX/DFX. The eluted solution was applied to HPLC under conditions as described by Gower *et al.* (1989).

Recovery of DFX and FX peaks was measured at 229 nm and 430 nm, respectively, and calculated from standards incubated and extracted as the samples.

Total iron was determined colorimetrically following ashing. Prior to ashing, 2 g tissues was weighed into tared Pyrex® ignition tubes in triplicate, and moisture content was determined after 24 h at 95°C. Sample tubes were transferred to a heating block and dry-ashed in a muffle furnace for 2 h at 250°C, followed by 20 h at 450°C. Four ml concentrated HNO₃ was added, and tubes were heated to 90-95°C in an aluminum foil-wrapped heating block on a temperature-controlled hot plate until dry. Wet ashing with HNO₃ was repeated. The entire dry/wet ashing procedure was repeated, where necessary, until a gray-white residue remained. Concentrated HCl (0.5 ml) was added to each tube. Tubes were vortexed, covered, and held at least 2 h. The volume was brought to 10 ml with distilled water. Two-ml sample aliquots were taken, and iron was measured colorimetrically as described for NHI. National Bureau of Standards bovine liver yielded 95% recovery.

Lipid analyses. Lipid measurements were made in duplicate using raw ground muscle from each sample, unless otherwise stated.

Lipids were extracted from the ground muscle samples by the method of Hara and Radin (1978), as modified by Willemot *et al.* (1985). The extracting solution (hexane: isopropanol, 3:2, v/v) contained BHT (0.01% w/v) to prevent sample autooxidation.

Total lipids were determined gravimetrically. Total phospholipids were quantified using dried lipid extracts taken up in hexane (3 ml hexane/100 mg lipid). Aliquots of 15 µl (0.5 mg fat) were transferred to the bottom of a screw-topped test tube (Pyrex, 15 ml) and dried under nitrogen. Phospholipids (mg) were quantitated by the ferrothiocyanate method

(Stewart, 1980) against a standard curve prepared with DL- α -phosphatidyl choline, dipalmitoyl (Sigma P-5911). Phospholipid content was expressed as % of total lipid, as well as % of wet tissue weight.

Fatty acid compositions of total lipid and phospholipid fractions were analyzed from 2 randomly selected pigs per treatment. 2.5 g of raw ground muscle was homogenized in 10 ml saline (0.9% NaCl in deionized water), and 0.1 ml ethanolic BHT (0.25%, w/v) for 10-15 seconds on setting 4 (Kinetmatica®, Model PT 10/35, Brinkman Instruments Co., Westbury, NY). Aliquots of the homogenate (0.8 ml) were transferred to each of 4 screw-topped test tubes, and lipids were extracted using the Bligh and Dyer method (1959). Phospholipids from the contents of 2 tubes were isolated on TLC plates (Krackeler, 5633-5) developed in a chloroform:methanol (8:1, v/v) solvent system. Phospholipid scrapings and dried total lipid (in remaining 2 tubes) were saponified/methylated by a modification of Metcalfe *et al.* (1966) with pentadecanoic acid (Fisher, EK-102-4876) as the internal standard. A megabore fused silica column (J & W Scientific, DB-23 series, 125-2332, 30 m, 0.75 mm ID, 0.20 μ m df) and 5710A Hewlett-Packard gas chromatography (GC) apparatus equipped to a 3390A Hewlett Packard integrator was used for the detection of fatty acid methyl esters (FAMES). GC conditions were as follows: injection temperature = 140°C; final oven temperature = 240°C; program rate = 8°C/minute; carrier gas = nitrogen; nitrogen flow rate = 30 ml/min. FAME standards (NuChek Prep, GLC-68B and GLC-79) were used to determine retention times. Fatty acids were calculated on a mole % basis.

Calcium and phosphorous in blood plasma were determined according to Bar *et al.* (1987).

Sensory evaluation

Eleven panelists were selected and trained after achieving at least a 75% accuracy rate in 4 consecutive-day triangle tests using samples of freshly cooked and 14-day or 16-day stored, cooked ground muscle. Training was conducted in ten 30-minute sessions over a two-week period using a group discussion format. Warmed-over aroma (WOA) was described using known quality descriptors summarized by Melton *et al.* (1987), and additional terms generated by the panel. Trainees rated WOA intensity in cooked, stored GP against a freshly cooked reference (labeled "R") using a 9-point scale (0=weak; 8=strong). Panelists were instructed to assign "0" or "1" to the reference as a means of calibration from day to day.

Cooked, stored ground muscle (~30 g) was spooned from each sandwich bag to 2 glass jars coded with 3-digit random numbers, topped with pre-cut aluminum foil liners, and screw-capped. A headspace of approximately 2 in was present in each jar. Jars were refrigerated at 4°C and warmed in a 70°C water bath 30 min prior to the panelists' arrival.

A randomized block design of 10 panelists and 15 pigs was used. Each of two panelists was paired with 3 pigs (one from each of the 3 treatment diets) and asked to evaluate 3 sets of samples on each day of testing. Each set contained 5 samples from the same pig stored as follows: reference (freshly cooked), 2, 4, 8 and 12 days. Format and ballot were the same as used during training. The sensory equipment was replicated on three alternate days. WOA scores were averaged from the two panelists'

triplicate replication (n=6) so that each pig had 5 scores which corresponded to days 0, 2, 4, 8 and 12.

α -tocopherol content (swine)

Under dim lighting, 2.5 g of pre-blended RF was homogenized in 7.5 g buffer (0.005 M Tris/Maleate, 0.001 M EDTA, 0.15 M KCl, pH 7.4, stored at 4°C) and 0.1 ml ethanolic BHT (0.25% w/v) in a 2 oz. plastic cylindrical vial (Capital Vial Inc., Fultonville, NY) on setting 4 for 10-15 seconds (Kinematica®, Model PT 10/35, Brinkmann Instruments Co., Westbury, NY). Homogenates (~1-2 ml) were transferred to plastic vials with rubber seal caps, flushed with nitrogen, capped, wrapped in aluminum foil, and stored at -70°C until further extraction. Homogenates (1 ml aliquots) were pipetted (in duplicate) into disposable glass screw-topped tubes, and 1 ml sodium dodecyl sulfate (SDS) (400 mM) was added. After vortexing, 2 ml ethanolic BHT (0.1% w/v) and 2 ml BHT (0.25% w/v) in heptane were added. Tubes were capped, vortexed for 2 minutes, and centrifuged (IEC Model HNS2, International Equipment Co., Needham Heights, MA) for 5 minutes at full speed. A known volume (~1.6 ml) of the upper (heptane) phase was recovered, placed in a glass conical centrifuge tube, and dried under nitrogen in a 30-40°F water bath. Exactly 200 μ l ethanol was added to each tube. Tubes were capped and vortexed for 10 seconds. Samples were filtered into vials compatible with Waters Intelligence Sample Processor (WISP) automatic sampler. Analyses were performed using high-performance liquid-liquid partition chromatography (HPLC) with a reverse-phase column described by McShane *et al.* (1991).

α -tocopherol content (turkey and chick)

We have developed a simple method for extraction of tocopherols from muscle tissues which shows a high extraction efficiency of the vitamin (data not shown) from muscle tissue, when this was compared with the method published by Burton *et al.* (1985). A 5-g sample of muscle tissue was thinly cut and frozen at -70°C . The tissues were freeze-dried and ground using a Waring blender (100 ml cup). α -Tocopherol from muscle powder (500 mg) was extracted with 4 ml ethanol containing 1% BHT, by using a Teflon homogenizer and a vortex mixer. The homogenate was centrifuged for 15 min at 5000 g. The supernatant was filtered through a $0.2\text{-}\mu$ membrane and $20\text{ }\mu\text{l}$ was injected to HPLC (LKB Broma), separated by a Merck Lichrocart column RP-18 $125\text{-}4\text{ }\mu\text{m}$, eluted by an isocratic mobile phase of methanol (flow 1 ml/min), and detected with an HPLC spectrofluorometer detector (Jasco FP 210), which was excited at 290 nm and emitted at 329 nm .

All the results are an average of three determinations for each sample. α -Tocopherol (Sigma, CO) was used for calibration of a standard curve.

DETERMINATIONS OF DEFENSE ENZYME (catalase, SOD, glutathione peroxidase)

Catalase activity was determined using an oxygen monitor (Yellow Spring Instrument Co., Model 53), as described by Kanner *et al.* (1991). This method is based on the ability of the oxygen electrode to quantitate O_2 generated from the decomposition of H_2O_2 by catalase. The test system contained 0.05 M buffer acetate, $\text{pH } 6.5$, 2 mM H_2O_2 and a $100\text{ }\mu\text{L}$

homogenate sample. The definition of a catalase unit is μmoles of O_2 generated in 1 ml/min.

Glutathione peroxidase (GSHPx) activity was determined using an HP 8452A spectrometer, as described by Del Maestro and McDonald (1985). This method is based on the oxidation of reduced glutathione (GSH) by GSHPx, coupled to the disappearance of NADPH by glutathione reductase (GSR).

SOD activity was determined by the pyrogallol autoxidation method, as described by Marklund (1985). The autooxidation of pyrogallol is highly dependent on O_2 and can be inhibited up to 97.5% by SOD.

The test system contained 50 mM potassium phosphate buffer, pH 7.0 with 0.5 mM diethylenetriaminepenta acetic acid (DTPA), 2 mM GSH, 1 U GSR, 0.6 mM t-butylhydroperoxide (t-BOOH), and 50 μl homogenate sample. The activity of GSHPx in the sample was determined by the change in NADPH oxidation per 3 min, as measured at 340 nm. One unit μg GSHPx causes the oxidation of 1 μmole GSH per minute in the system.

The test system contained 50 mM buffer Tris-HCl, pH 8.2 with 1 mM DTPA, 10 μl catalase (100 $\mu\text{g}/\text{ml}$ Sigma, 0.2 mM pyrogallol in 10 mM HCl, and 100 μl homogenate sample. SOD activity was determined by the inhibition of pyrogallol autooxidation every 3 minutes, as measured at 420 nm using an HP8452A recording spectrophotometer. One unit of SOD causes 50% inhibition of pyrogallol autooxidation.

Statistics (swine)

A split-plot design with each pig as the experimental unit was used as described by Miller *et al.* (1993). Outliers were excluded, based on residual plots. Individual grand means (GMs) for each pig (average of days 0, 2, 4, 8 and 12) were groups according to treatment, and differences between treatments were determined by one-way analysis of variance (ANOVA) and two-sample independent t-tests with $\alpha=0.05$. Statistical analyses were conducted using MINITAB Statistical Software (State College, PA), as outlined in Miller *et al.* (1993).

The experiment followed a split-plot design with each pig representing one experimental unit, dietary iron as the whole plot factor, and storage time and bags of pork as subplot factors. MINITAB Statistical Software (State College, PA) was used for all analyses.

Single-day data. Means and standard error of the mean (SE) were calculated for each pig, and outliers were excluded on the basis of residual plots. Data were separated according to the 3 iron treatments ($n=5$ values per treatment, unless otherwise stated), and analyzed by analysis of variance (ANOVA) and independent two-sample t-tests when ANOVA was significant ($p<0.05$). Regression of analysis of chemical and/or sensory data was used to detect linear trends or quadratic fits as a function of iron level or iron level squares, respectively ($p<0.05$).

Over-time data. Treatment data from the 12-day storage study was analyzed for time effects and absolute differences. Slopes (SLP) of NHI, TBARS, and WOA plotted against storage days were calculated using

the best linear transformation of the x-axis [$\log_{10}(\text{days} + 1)$, $\sqrt{(\text{days})}$, or $(\text{days} + 1)^{-1}$]. Absolute differences between pigs were corrected for time by averaging the 5 chemical or sensory values for days 0, 2, 4, 8 and 12 for each pig (individual grand mean (IGM)). Treatment grand means (TGMs) were also computed. SLPs and IGMs were sorted by dietary iron treatment level and analyzed by ANOVA, t-tests, and regressions as described under single-day data.

Correlations. Three separate methods were used for each of the following correlations: TBARS vs. WOA, TBARS vs. NHI, and NHI vs. WOA. First, the factor of time was removed by regressing IGMs to show between-animal relationships. Second, within-animal deviations (IGM minus single-day response) were correlated, revealing relationships over time, while removing animal-to-animal variations. Thirdly, correlations through time were made by using the 5 single-day data points for each animal, leaving animal-to-animal variation uncorrected.

Statistical analysis (turkeys, chicks)

ANOVA, simple and factorial, and calculation of regression were carried out according to Snedecor and Cochran (1967). The main factors tested in the turkeys experiment were Fe and vitamin E levels (Experiments 2 and 5), minerals (Fe or Cu) and vitamin levels (Experiment 3), and Fe-dextran injection and vitamin E level (Experiment 4). The main factors tested in the chick experiment were vitamin D and Fe level (Experiment 3). Difference among means were determined by the multiple range test (Duncan, 1955). The data on body weights and the concentrations of hemoglobin, calcium and phosphorous in blood obtained during the

course of the experiments are of those birds which were slaughtered, either during the experiments or at their termination.

RESULTS AND DISCUSSION

Growth and hematology (swine)

Swine fed high-iron diets had slightly higher mean final weights than pigs from the medium- and low-iron groups (Table 4). However, SI, %TS, Hb, %PCV, and PT were not affected by dietary iron treatment (data not shown). No signs of iron-deficiency were evident.

TABLE 4. Initial and final pig weights ($\pm$ SE)

Treatment	Initial (kg)	Final (kg)
LOW Fe	25.0 $\pm$ 0.3	98.5 $\pm$ 3.9 ^{ab}
MEDIUM Fe	24.5 $\pm$ 0.7	100.3 $\pm$ 1.3 ^a
HIGH Fe	24.6 $\pm$ 0.7	106.1 $\pm$ 1.0 ^b

^aFinal live weight treatment means with different superscripts differ ($p < 0.05$). Slope of final weights ($n=14$) regressed against dietary iron was significant ($p < 0.05$).

Chemistry

Both NHI and TBARS increased significantly during the 12-day storage period (Figs. 1 and 2). These data suggest that some disruption of the porphyrin rings occurs during storage, especially in the early stages. Iron released from the heme complex as a result increases NHI concentration and this may have increased TBARS formation.

However, TBARS in freshly cooked GP (day 0) and pork stored for 4 and 12 days did not differ between treatments, while NHI treatment

differences were significant on all 5 storage days. The increases in NHI and TBARS were more pronounced in pork from the high-iron treatment group (Figs. 1 and 2).

FIG. 1. Effect of storage and dietary iron treatment on TBARS ($\pm$ SE) in cooked ground pork. [* = significant difference ($p < 0.05$) between treatment means ($n=4$) at storage day. Regression analysis of transformed slopes showed higher ($p=0.006$) rates of TBARS increase over time as a function of increasing dietary iron levels].

Treatment level	TBARS TGM ^a (μ g MDA/g)
LOW Fe	3.761 ± 0.045^b
MEDIUM Fe	3.791 ± 0.052^b
HIGH Fe	4.210 ± 0.068^c

^aTreatment grand mean (TGM) = a mean for each pig was calculated by averaging TBARS from 2 storage days. TGM is the mean of all pigs in the same treatment.

^bTGMs with different superscripts differ ($p < 0.05$).

FIG. 2. Effect of storage and dietary iron treatment on NHI ($\pm$ SE) in cooked ground pork. [* = significant difference ($p < 0.05$) between treatment means ($n=4$) at storage day. Regression analysis of transformed slopes showed higher ($p=0.014$) rates of NHI increase over time as a function of increasing dietary iron levels].

Treatment level	NHI $\pm$ TGM ^a (μ g/g)
LOW Fe	4.981 ± 0.203^b
MEDIUM Fe	4.838 ± 0.062^b
HIGH Fe	6.297 ± 0.209^c

^aTreatment grand mean (TGM) = a mean for each pig was calculated by averaging NHI from 5 storage days. TGM is the mean of all pigs in the same treatment.

^bTGMs with different superscripts differ ($p < 0.05$).

TI in freshly cooked GP was also higher in the high-iron group (Fig. 3). A significant quadratic trend in muscle TI ($p=0.029$) as a function of dietary iron level squared was observed, suggesting a threshold for the relationship between muscle iron concentration and dietary iron. Above this threshold, muscle TBARS, NHI and TI increased, but below the threshold dietary iron concentration did not affect tissue iron or TBARS.

It is well established that homeostatic regulation of total body iron operates at the site of absorption: iron-deficient animals absorb a higher percentage of dietary iron than iron-adequate animals. Apparently this regulation was partially overridden in the high-iron group since tissue iron concentrations were greater in this group.

A threshold was also evident for liver TI and NHI (Fig. 3). Both muscle and liver from the low- and medium-iron fed swine did not differ in TI and NHI concentration. Hence, the low- and medium-iron treatment diets appeared to be in the range where homeostasis was operating successfully.

Liver TI and NHI correlated with the respective TI (day 0) and NHI (IGMs) concentrations in ground muscle (Table 5). Our data are direct evidence that changes in iron status are reflected in muscle iron concentrations.

Clearly, swine rations contain a large range of iron levels. However, few analyzed values have been reported. We analyzed rations from one producer and from the Cornell Swine Farm and found total dietary iron to vary between 302-737 ppm (Miller, 1992). This is in agreement with published analyses of 89 swine diet samples formulated by swine

FIG. 3. Effect of dietary iron on iron in freshly cooked muscle (day 0) GP and in raw liver. Values are means $\pm$ SE. [Treatment bars for GP TI (n=4) and GP NHI (n=4) with different superscripts differ ($p<0.05$). Liver NHI (n=5, $p=0.024$) increased with respect to dietary iron level according to linear regression analysis].

producers (Ewan, 1985, 1986) and of a basal diet fed by Pond and co-workers (1989). Even though we approximated our high-iron treatment diet to contain an iron level representative of that used by commercial producers, it probably was formulated at a lower iron level because of special precautions taken by us with respect to cleanliness when mixing diets and precision when weighing micronutrients.

TABLE 5. Correlation coefficients

Relationship	r^a
Liver TI vs. Muscle TI ^b	0.816
Liver NHI vs. Muscle NHI ^c	0.643
TBARS vs. NHI ^d	0.901
TBARS vs. WOA ^e	0.903
NHI vs. WOA ^f	0.754

^an=12 pigs for all correlations except n=10 pigs for TBARS vs. NHI ($p < 0.05$).

^bMuscle TI from day 0 and liver TI at time of slaughter (independent of time correlation).

^cMuscle NHI from GM of 5 storage days for each pig (independent of time correlation).

^dGMs of 5 storage days for each pig (independent of time correlation).

^e5 values for each pig (over-time correlation).

^f5 values for each pig (over-time correlation).

TABLE 6. Total lipid and phospholipid content of raw ground pork ($\pm$ SE)

Treatment level	Total lipid (%)	Phospholipid (% lipid) ^a	Phospholipid (% wet tissue) ^b
LOW Fe	13.53 $\pm$ 2.09	2.92 $\pm$ 0.49	0.36 $\pm$ 0.04
MEDIUM Fe	13.21 $\pm$ 1.64	3.01 $\pm$ 0.35	0.37 $\pm$ 0.02
HIGH Fe	12.84 $\pm$ 2.04	3.48 $\pm$ 0.31	0.42 $\pm$ 0.04

^amg phospholipid/100 mg lipid,

^bmg phospholipid/100 mg wet tissue.

In our study, differences in pork lipid peroxidation due to dietary iron treatment were not as dramatic as Kanner *et al.* (1990) observed in turkey. This may be partially due to the precautions mentioned above. Turkey is also higher in polyunsaturated fatty acids, such as arachidonic acid, and total phospholipids when compared to pork (Ang, 1988; Wilson *et al.*, 1976).

Thus, WOF and lipid oxidation in general have been more problematic in poultry than in red meat (Wilson *et al.*, 1976).

Dietary iron treatment did not affect FAMES of total lipid or phospholipid fractions of raw GP (data not shown). Raw pork lipid content varied from 8-20% between pigs, but no differences were observed due to dietary iron treatment. This wide range of total fat percentage was expected due to similar variability in tenth rib backfat between animals.

Phospholipid content was slightly higher in the high-iron treatment group, although the difference was not significant (Table 6). Since GP was prepared with both intramuscular fat and adipose tissue, phospholipid content was lower than we found in isolated muscle. Phospholipids are present in cell membranes. Because red muscle fibers contain more mitochondria and cell membranes than do white muscle fibers (Forrest *et al.*, 1975), phospholipid concentration is higher in intact muscle as we reported in the following paper (Miller *et al.*, 1993).

Warmed-over aroma

The warmed over aroma (WOA) was rated instead of WOF for the general safety of the panel and for sensitivity reasons. Cooked meats held at refrigeration temperature become rancid after 8 days (Anon., 1984) and may be unpalatable. The human nose is sensitive to oxidation by-products commonly found in cooked, refrigerated meats (Dupuy *et al.*, 1988; Frazzolari, 1978). Other researchers have also reported meat aroma responses rather than flavor scores (Poste *et al.*, 1986; Tellefson *et al.*, 1982).

Trained panelists successfully detected WOA in cooked GP throughout the storage study (Fig. 4). On storage day 4, sensory scores reached a plateau which corresponded with the plateau of MDA production (Fig. 1). Unlike TBARS, however, the panel found no differences in WOA intensity among the three treatments. Our results suggested that manipulating dietary iron levels in swine at or below 209 ppm may have reduced lipid oxidation but not at consumer-perceivable levels.

FIG. 4. Effect of storage and dietary iron treatment on WOA sensory scores ($\pm$ SE) for cooked ground pork. [No significant differences were found between treatment means ($n=5$) on any storage day, between rates of WOA increase, or between IGMs corrected for time ($p>0.05$)].

Treatment level	WOA $\pm$ SE ^a (Grand mean)
LOW Fe	3.34 $\pm$ 0.13
MEDIUM Fe	3.49 $\pm$ 0.19
HIGH Fe	3.63 $\pm$ 0.13

^aTreatment grand mean (TGM) = a mean for each pig was calculated by averaging WOA from 5 storage days. TGM is the mean of all pigs in the same treatment.

Failure to detect differences may be explained by the manner in which panelists were trained. The panel as a whole utilized only 3/4 of the WOA intensity scale on the sensory ballot. During training and actual testing, the most intense WOA samples were stored for 12 days. However, panelists were pre-selected based on triangle tests where freshly cooked and 14- or 16-day stored pork was used. Thus, panelists may have been influenced by their earlier experiences in the pre-screening and, consequently, reserved the upper-end of the scale for samples of comparably high intensities. Unjudged stimuli affect judgment scales in sensory tests by anchoring the scale at either end (Sarris and Parducci, 1978).

Another plausible explanation for the panel's inability to distinguish differences in WOA between treatments may relate to Weber's Law. In short, as the stimuli intensity increases, a large incremental change in concentration is required for subjects to detect a just noticeable difference (Stevens, 1971). Hence, in our study the incremental change in WOF concentration between days 4 and 12 may have been too small for panelists to detect a difference. In addition, panelists may have suffered biases involving adaptation, saturation, or fatigue of the nasal passages. Poste *et al.* (1986) reported difficulty in their panelists' abilities to rate WOA in cooked pork after 8 days of storage because of rancidity odor overlap. Perhaps our panel also confused WOA with rancidity, thus rating WOA the same in cooked pork stored for 4 or more days.

Tarladgis *et al.* (1960) reported a TBARS threshold of 0.5 to 1.0 μg MDA/g for detection of WOA in pork by trained panelists. However, TBARS values vary by method despite high correlations. For example,

the distillation method (Tarladgis *et al.*, 1960) produced higher TBARS than the extraction technique (Melton, 1983). Theoretically, since we used a modification of the Witte *et al.* (1970) extraction method, our TBARS threshold may have been slightly lower than Tarladgis' range. Our TBARS values for cooked GP were higher than 1 µg MDA/g on day 2 and after. Therefore, according to Tarladgis' TBARS threshold range, our panelists should have detected WOA in all cooked GP treatments at day 2 and beyond.

Since trained panelists were unable to recognize WOA differences according to dietary iron treatment, withdrawing iron supplements from swine rations to protect against lipid oxidation and WOA may be impractical.

Correlations

Significant correlations are shown in Table 5. Muscle NHI correlated significantly with TBARS and, to a lesser extent, with WOA. Since our trained panelists failed to detect differences in WOA among the three treatment groups, WOA did not significantly correlate with TBARS or NHI when the factor of time was removed. However, strong through-time correlations were found between all three variables. Our study confirmed that chemical tests, such as TBARS and NHI, were valuable indicators of WOA over time. In fact, not only were these chemical measurements more applicable for large numbers of stored samples, but they may also be more sensitive, consistent, reliable, and economically feasible than a trained consumer panel. For the practical measure of product quality and off-aroma, however, there is no replacement for the sensory panel.

NHI and TI in muscle

Increasing dietary iron in swine rations during the last 13 weeks prior to slaughter did not affect hematological iron status indicators and only slightly improved growth (Miller *et al.*, 1993). Nonetheless, NHI concentrations ($\mu\text{g/g}$ wet wt.) in LD and RF increased with dietary iron level, except in raw RF (Table 7). LD and RF were studied because they are classified as white (<30% red fibers) and red (>40% red fibers) porcine muscles, respectively (Beecher *et al.*, 1965). RF contained significantly more TI (Fig. 5) and NHI (Table 7) than did LD.

Previously, we reported that cooking increased NHI in meats and speculated that the increase was due to oxidative cleavage of the porphyrin ring, allowing release of the heme iron (Schricker and Miller, 1983). In this study, however, NHI did not significantly differ between raw and freshly cooked pork muscles (Table 7). Since pork was cooked in vacuum-sealed oxygen-impermeable bags, oxidative cleavage of the porphyrin ring may have been limited by low oxygen concentration. The short cooking time (~15 min) may also have been a factor.

TABLE 7. Changes in NHI and TBARS immediately after cooking LD and RF muscles ($\pm$ SE)^a

Muscle	Treatment	NHI (μ g/g)		TBARS (μ g/g)	
		Raw	Cooked	Raw	Cooked
LD	LOW Fe	1.81 $\pm$ 0.18	2.08 $\pm$ 0.19 ^{de}	0.04 $\pm$ 0.14	0.05 $\pm$ 0.01 ^e
	MEDIUM Fe	2.02 $\pm$ 0.17	2.14 $\pm$ 0.09 ^d	0.04 $\pm$ 0.01	0.10 $\pm$ 0.02 ^f
	HIGH Fe	2.50 $\pm$ 0.33	2.78 $\pm$ 0.21 ^e	0.03 $\pm$ 0.01	0.10 $\pm$ 0.02 ^{ef}
	Pooled ^b	2.11 $\pm$ 0.15	2.39 $\pm$ 0.14	0.04 $\pm$ 0.01	0.09 $\pm$ 0.01 ^h
	p ^c	0.05	0.02	N.S.	N.S.
RF	LOW Fe	3.53 $\pm$ 0.44	3.64 $\pm$ 0.32 ^{fg}	0.04 $\pm$ 0.02	0.11 $\pm$ 0.02
	MEDIUM Fe	3.92 $\pm$ 0.58	3.63 $\pm$ 0.18 ^f	0.04 $\pm$ 0.01	0.15 $\pm$ 0.01
	HIGH Fe	4.40 $\pm$ 0.25	4.95 $\pm$ 0.48 ^g	0.03 $\pm$ 0.01	0.11 $\pm$ 0.02
	Pooled ^b	3.95 $\pm$ 0.26 ⁱ	4.07 $\pm$ 0.25 ⁱ	0.04 $\pm$ 0.01	0.12 $\pm$ 0.01 ^{hi}
	p ^c	N.S.	0.02	N.S.	N.S.

^an=15 pigs for each muscle analysis except n=12 for Cooked LD (NHI and TBARS). Muscles analyzed in triplicate for each pig (μ g/g wet wt.).

^bPooled means for all three treatments using each pig mean.

^cSignificant linear trend with increasing dietary Fe level (p<0.05). N.S.=trend not significant.

^{defg}Treatment means in the same column for a given muscle marked with different superscripts differ (p<0.05).

^hSignificantly different from raw pooled mean (NHI or TBARS) in same row (p<0.05).

ⁱSignificantly different from LD pooled mean in same column (p<0.05).

NHI increased gradually in cooked LD and RF during storage at 4°C (Fig. 6). Since cooked pork was stored in oxygen-permeable bags, oxidative cleavage of the porphyrin ring may have occurred. NHI release in meats appears to be dependent on time, temperature, and oxygen presence during cooking (Buchowski *et al.*, 1988; Han *et al.*, 1993; Schricker *et al.*, 1982).

Low- and medium-iron fed pigs exhibited similar muscle TI levels. The same pattern was observed in liver TI and NHI, as well as in cooked ground pork NHI (Miller *et al.*, 1993). It appears that iron absorption was

down-regulated in the medium-iron group since total dietary iron was double that of the low-iron group but muscle TI levels were similar.

FIG. 5. Effect of dietary iron on mean TI ($\pm$ SE) in freshly cooked LD and RF (day 0). [RF TI regressed against dietary Fe was linearly significant ($p < 0.05$). Treatment means ($n=5$) within a muscle type were not different ($p > 0.05$)].

NHI in liver has long been used as an indicator of iron status. As in our previous paper (Miller *et al.*, 1993), we found that muscle NHI and TI were significantly associated with liver NHI and TI, respectively (Table 8).

FIG. 6. Effect of storage and dietary iron treatment on NHI ($\pm$ SE) in cooked LD and RF. [* = significant difference ($p < 0.05$) at storage day between treatment means. LD slopes and TGMs^a regressed against dietary iron and dietary iron squared showed significant ($p < 0.05$) linear and quadratic trends, respectively].

Treatment level	LD NHI TGM ($\mu\text{g/g}$)	RF NHI TGM ($\mu\text{g/g}$)
LOW Fe	2.79 ± 0.12^{bc}	5.09 ± 0.25^{de}
MEDIUM Fe	2.52 ± 0.14^b	4.70 ± 0.38^d
HIGH Fe	3.12 ± 0.15^c	6.21 ± 0.49^e

^aTreatment grand mean (TGM) = a GM mean for each pig was calculated by averaging NHI from 5 storage days. TGM is the mean of all pigs in the same treatment.

^{bcd}e TGMs in the same column with different superscripts differ ($p < 0.05$).

Lipid oxidation in cooked pork

Dietary iron did not affect TBARS in raw or freshly cooked (day 0) RF or raw LD and only slightly influenced TBARS in cooked LD (Table 7). Yet cooking increased TBARS in both muscles.

Based on slopes and GMs, cooked RF stored at 4°C for 12 days oxidized faster than cooked LD (Fig. 7). Since both TI and NHI were higher in RF, muscle iron concentration may have been responsible for the more rapid oxidation in the RF. Higher total phospholipids in the RF also may have enhanced TBARS formation (Table 9). Since red muscle is composed of more fibers per unit area, it contains more cell membranes rich in phospholipids. RF contained less total lipid than LD. Although red muscle usually is higher in fat than white muscle, Beecher *et al.* (1965) reported that RF was lower in lipid content than other porcine red muscles. In general, our values for phospholipids and total lipids agreed with literature reports (Morrison and Campbell, 1971; Wilson *et al.*, 1976).

NHI and TBARS were correlated when the time factor was removed (Table 8). These data agreed with our previous findings in cooked ground pork (Miller *et al.*, 1993).

Although TBARS were higher in LD and RF from the high-iron group, the effect was not as great as we reported for cooked ground pork from these same pigs (Miller *et al.*, 1993), nor as Kanner *et al.* (1990) observed for turkey.

FIG. 7. Effect of storage and dietary iron treatment on TBARS ($\pm$ SE) in cooked LD and RF. [* = significant difference ($p < 0.05$) at storage day between treatment means. LD and RF slopes and TGMs^a each regressed against dietary Fe showed significant ($p < 0.05$) linear trends].

Treatment level	LD TBARS TGM ($\mu\text{g/g}$)	RF TBARS TGM ($\mu\text{g/g}$)
LOW Fe	1.76 ± 0.08^b	2.89 ± 0.08^d
MEDIUM Fe	1.80 ± 0.05^b	3.00 ± 0.04^e
HIGH Fe	2.04 ± 0.06^c	3.17 ± 0.06^e

^aTreatment grand mean (TGM) = a GM mean for each pig was calculated by averaging TBARS from 5 storage days. TGM is the mean of all pigs in the same treatment.

^{bcd}e TGMs in the same column with different superscripts differ ($p < 0.05$).

TABLE 8. Correlation coefficients (r)^a

Relationship tested	r
Liver TI vs. LD TI ^b	0.539
Liver NHI vs. LD TI ^b	0.681
Liver NHI vs. LD NHI ^c	0.633
Liver NHI vs. RF NHI ^c	0.634
LD TBARS vs. RF NHI ^d	0.624
RF TBARS vs. LD NHI ^d	0.728

^an=14 pigs for all correlations except n=13 pigs for LD TBARS vs. LD NHI and n=15 pigs for liver NHI vs. RF NHI. All correlations were independent of time (p<0.05).

^bTI (LD and RF) measured on day 0.

^cNH (LD and RF) taken from GM of 5 storage days for each pig.

^dTBARS (LD and RF) and NHI (LD and RF) taken from GM of 5 storage days for each pig.

TABLE 9. Total lipid and phospholipid content of raw ground pork (±SE)

Muscle	Treatment	Total Lipid (%)	Phospholipid (% lipid) ^a	Phospholipid (% wet tissue) ^b
LD ^c	LOW Fe	3.11 ± 0.45	11.28 ± 1.66	0.32 ± 0.02
	MEDIUM Fe	2.47 ± 0.29	14.49 ± 2.69	0.33 ± 0.02
	HIGH Fe	2.35 ± 0.24	14.58 ± 1.66	0.33 ± 0.02
	Pooled ^d	2.64 ± 0.20	13.45 ± 1.17	0.33 ± 0.02
RF ^c	LOW Fe	1.71 ± 0.26	25.92 ± 2.87	0.41 ± 0.02
	MEDIUM Fe	1.57 ± 0.16	29.18 ± 3.07	0.44 ± 0.02
	HIGH Fe	1.92 ± 0.14	26.65 ± 3.63	0.49 ± 0.04
	Pooled ^d	1.73 ± 0.11 ^e	27.25 ± 1.75 ^e	0.45 ± 0.02 ^e

^amg phospholipid/100 mg lipid.

^bmg phospholipid/100 mg wet tissue.

^cn=15 pigs for each muscle (in duplicate). Columns within each muscle were not different (p>0.05).

^dPooled means for all three treatments using each pig GM.

^eSignificantly different from LD pooled mean in same column (p<0.05).

α -tocopherol in RF

RF was analyzed for α -tocopherol because higher concentrations were shown in dark pork muscle than in white muscle (Yamauchi *et al.*, 1980). Although α -tocopherol in RF did not change immediately after cooking, significant degradation occurred in both raw and cooked RF stored at 4°C (Table 10). A fat-soluble vitamin present in cell membranes, vitamin E is unstable to heat, light and oxygen. Because pork was stored in oxygen-permeable packages (polyvinyl chloride=raw pork; polyethylene=cooked pork), tocopherol content was expected to decrease. Cooking predisposed pork to faster α -tocopherol losses in a shorter storage period.

No differences in plasma or RF α -tocopherol were detected between treatments, perhaps because dietary tocopherol was similar in all three treatment diets (Miller *et al.*, 1993). Cooked RF on day 12 revealed a faster rate of tocopherol degradation in RF from the high-iron group, suggesting the need for more α -tocopherol to quench free radicals in lipid membranes from the high-iron treatment. Vitamin E is destroyed *in vivo* when excess iron is consumed (Lannek *et al.*, 1962).

The extent of lipid oxidation in cooked pork appeared to differ with different muscle cuts. Dark porcine RF muscle oxidized at a faster rate than light porcine LD muscle. A threshold range was also apparent in both muscle and liver NHI and TI and in muscle TBARS, in response to dietary iron level. Hence, dietary iron may influence NHI and oxidative stability in cooked meats. Future work may include feeding higher amounts and a wider range of dietary iron (100-1000 ppm) to swine, since our diets were formulated at lower iron levels than industry norms.

TABLE 10. α -Tocopherol (ng/g) in raw and cooked RF muscles stored at 4°C (mean $\pm$ SE)^a

	Treatment	Day 0	Day 16
Raw	LOW Fe	438.7 $\pm$ 21.6	384.2 $\pm$ 21.7
	MEDIUM Fe	379.6 $\pm$ 63.2	260.8 $\pm$ 47.5
	HIGH Fe	434.4 $\pm$ 27.0	326.0 $\pm$ 25.7
	Pooled ^b	417.6 $\pm$ 23.4	323.7 $\pm$ 22.4 ^e
	p ^c	N.S.	0.04
	Treatment	Day 0	Day 12
Cooked	LOW Fe	459.2 $\pm$ 34.5	33.1 $\pm$ 20.5
	MEDIUM Fe	370.7 $\pm$ 29.7	4.6 $\pm$ 4.6
	HIGH Fe	453.1 $\pm$ 32.9	0.0 $\pm$ 0.0
	Pooled ^b	427.6 $\pm$ 20.4	12.6 $\pm$ 7.6 ^{de}
	p ^c	0.05	N.S.

^an=15 pigs for each muscle and storage period. n=5 pigs per treatment.

^bPooled treatment mean using each pig GM.

^cSignificant quadratic trend with increasing dietary Fe level squared. N.S.=trend not significant. Treatment means within each muscle for the same storage period were not different (p>0.05).

^dSignificantly different from raw pooled mean in same column (p<0.05)

^eSignificantly different from day 0 pooled mean in same row (p<0.05).

ROLE OF DIETARY VITAMIN E AND ASCORBIC ACID ON FE-INDUCED *IN VIVO* LIPID PEROXIDATION IN SWINE IN STORED PORK

Results from our research demonstrated that manipulation of dietary iron levels in swine rations is unlikely to significantly impact WOF in pork. However, the results did show that modest increases in dietary iron increased tissue iron stores (non-heme iron) and that lipid peroxidation in stored pork, as measured by TBARS, correlated with tissue non-heme iron. These results raise important questions about a possible relationship between tissue iron stores and oxidative stress in animals including humans. Thus, we conducted a second experiment to address the following questions:

- (1) Do moderate elevations in tissue storage increase lipid peroxidation *in vivo*?
- (2) Will supplementation of the diet with vitamin E and/or C reduce or prevent increased lipid peroxidation caused by elevated tissue iron stores?
- (3) Will supplementation of the diet with vitamin E and/or C increase storage stability of raw and cooked pork from animals moderately loaded with iron?

Animals and housing

Thirty gilts from the Cornell swine herd were identified at birth. Six received 100 mg iron given as a single injection of iron dextran by the

time they were 3 ± 1 day old. Twenty-four received 7 injections of iron dextran (200 mg Fe per injection) at 5-day intervals beginning at age 3 ± 1 day of age.

The piglets were housed with their sows for 3 weeks in commercial metal farrowing crates with wire-mesh floors. They had access to sow's milk, water and a pelleted creep feed. They were weaned at approximately 3 weeks of age and transferred to a group pen where they had *ad libitum* access to water and creep feed. At age 4 weeks, the piglets were transferred to individual stainless steel cages with mesh floors, automatic waterers, and plastic creep feeders. At age 7 the pigs were transferred to individual stalls with concrete floors, automatic waterers, and wooden feeders for the remainder of the study.

Experimental design

At age 4 weeks, the pigs were allocated to 5 treatment groups, divided into 3 blocks of 10 pigs each. Blocking was necessary because our temperature-controlled swine facility could accommodate only 12 cages at a time. For each block, the 2 pigs that received 100 mg injected iron were put in one group, hereafter designated the control group. The remaining pigs were allocated to 4 groups of 2 pigs, each so that the mean body weight of all groups were approximately equal. These 4 groups were randomly assigned to treatment. The 3 blocks were run sequentially, i.e. after completing the feeding experiment with block 1, selection and feeding of the second block was begun. When block 2 was finished, block 3 was begun.

Treatments consisted of feeding diets with varying amounts of supplemental vitamin E and/or vitamin C for 8 weeks (Table 11). The control treatment and the Fe-only treatment were fed the same diet. All diets were formulated to meet or exceed NRC recommendations (NRC, 1988) for growing swine (Table 12). The pigs were fed a "weaner" ration for the first 3 weeks of the feeding trial and a "grower" ration for the remaining 5 weeks.

TABLE 11. Treatment groups

	Injected Fe (mg)	Vitamin C (mg/kg)	Vitamin E (IU/kg)
Control	100	0	
Fe-Only	1400	0	0
Fe + C	1400	2000	0
Fe + E	1400	0	200
Fe + E + C	1400	2000	200

Pigs were offered a quantity of feed equal to the NRC estimate for "expected feed intake" (NRC, 1988) plus 15%. Most animals cleaned up their feed each day. Pigs were weighed every other day and blood samples were taken from the anterior vena cava the day prior to the beginning of the feeding trial, and at 2-week intervals throughout the trial. At the end of the feeding trial, animals were slaughtered according to traditional practices. Livers were removed, perfused with isotonic saline, and frozen at -20°C. Following a 24-hour carcass chill at 4°C, hams were excised, trimmed to ~0.32 cm fat, boned, and ground in a stainless steel bowl chopper. The ground pork was divided into 100±5 g lots, packaged

in oxygen impermeable pouches, vacuum-sealed, and stored at -20°C until further analysis.

TABLE 12. Composition of unsupplemented weaner and grower diets¹

Ingredient (%)²	Weaner (wks 1-3)	Grower (wks 4-8)
Corn ³	46.179	75.198
Soybean meal ⁴	27.965	22.267
Dried whey ⁵	20.000	---
Corn oil ⁶	3.001	---
Mono/Dicalcium PO ₄ ⁷	1.069	0.871
Limestone ⁸	0.724	0.782
Salt ⁹	0.543	0.532
Antibiotic ¹⁰	0.250	---
Vit./Min. Premix ¹¹	0.250	0.100
Lysine ¹²	0.019	0.100
	100.000%	100.000%
Iron (mg/kg) ¹³	209.2 ± 12.9	204.9 ± 13.8

¹Weaner ration was fed from weeks 1-3. Grower ration was fed from wks 4-8.

²By formulation.

³Shelled yellow; 8.2% protein, 0.03% Ca, 0.28% P, 0.003% Fe.

⁴Dehulled; 48% protein, 0.26% Ca, 0.64% P, 0.016% Fe.

⁵USPH Grade A Spray Dry Sweet Whey (Leprino Foods, Denver, CO); >11% protein, ≤1% crude fiber, >1% crude fat.

⁶Product #014-250 (Archer Daniels Midland, Decatur, IL).

⁷Nutrena® (Cargill, Minneapolis, MN); >15% Ca, >20.5% P, 1.29% Fe, ≤0.205% Fl.

⁸Limecrest® (Limestone Products Corp., Sparta, NJ); >38% Ca, 0.0144% Fe.

⁹Iodized (Agway, Ithaca, NY).

¹⁰Chlorotetracycline-50 Type A Medicated Article (Carl S. Akey, Inc., Lewisburg, OH); 50 g chlorotetracycline/1b.

¹¹Swine Premix (Carl S. Akey, Inc., Lewisburg, OH); >3.2% Fe (as ferrous sulfate), >0.4% Cu, >0.012% Se, >7920 IU/kg vitamin E.

¹²(Agway, Ithaca, NY); >98.5% L-lysine monohydrochloride, >78.8% L-lysine

¹³By analysis, n=9 for each diet.

***In vivo* lipid peroxidation**

Iron status was assessed prior to slaughter by measuring hemoglobin concentration and transferrin saturation. Iron stores were assessed by measuring non-heme and total iron in the livers taken at slaughter. Vitamin E status was assessed by measuring α -tocopherol serum, liver, and muscle (we have not yet completed the vitamin E analyses).

Thiobarbituric acid reactive substances (TBARS) in serum and liver and red blood cell fragility (RBC fragility) were used as indicators of oxidative stress.

Pork quality

Color stability in raw ground pork was assessed, by recording color values using a Gardner Color Difference Meter (Model AC-2A, Gardner Instruments) daily for 12 days. During this time the raw samples were wrapped in PVC film and stored at 4°C under soft white fluorescent lighting.

Peroxidation in cooked pork was monitored by TBARS. The frozen samples were thawed, cooked in an 80°C water bath for 25-30 minutes, and stored in polyethylene bags at 4°C. Samples were withdrawn for analysis on days 0, 2, 4, 6, 8 and 10, where day 0 is the day the samples were cooked. Non-heme iron was also measured each day. Total iron was measured on day 0 and assumed constant during storage.

Growth

Animals grew normally and there were no differences in weight gain or feed efficiency between groups.

Iron status

The animals given the iron injections had higher transferrin saturations and hemoglobin concentrations at week 0 of the feeding trial than the control (Fig. 8). These differences disappeared by the 2nd or 4th week. The lower initial values for the control animals suggests that they were iron-deficient even though they received the recommended iron injections and iron-supplemented diets that are commonly used in the U.S. swine industry. By the 4th week, all groups had similar values for hemoglobin and transferrin saturations. The normal transferrin saturation values for hemoglobin and transferring saturations. The normal transferring saturation values in the injected animals is evidence that they were not severely iron loaded.

As expected, liver total and non-heme iron were significantly elevated in the animals that received the additional iron injections (Fig. 9). An unexpected finding was that the groups that received supplemental vitamin E in their diets had lower liver iron stores compared with the groups that did not get supplemental vitamin E.

Oxidative stress

Liver TBARS were elevated in the animals that received iron injections (Fig. 10). Vitamin C reduced the effect, Vitamin E and Vitamin E + C markedly inhibited the effect. Values for RBC hemolysis were elevated on day 0 for groups 2, 3, 4 and 5, but the effect disappeared after 2 weeks (Fig. 11).

Pork quality

Color stability (monitored by recording a-value, an indicator of redness) in treatment Fe + E + C was significantly better than color stability in the other treatments. Vitamin E supplementation significantly reduced TBARS development in the cooked pork (Fig. 12). TBARS and non-heme iron were correlated in the cooked pork ($r=0.797$).

Iron injection is a convenient way to elevate body iron stores in swine. The data (transferrin saturation and liver iron) show that the animals had elevated stores but that the elevation was not enough to cause an increase in transferrin saturation after week 0 of the feeding trial. This indicates that the animals were not severely overloaded. Animals suffering from severe iron overload may have transferrin saturation values approaching 100%. The significant effect of vitamin E on iron stores in the liver is a surprising finding and we have no explanation for the observation. Three mechanisms are possible: (1) Vitamin E inhibited absorption of iron from the diet; (2) Vitamin E increased iron losses; or (3) Vitamin E affected the distribution of iron in the body. We are unaware of any data that would confirm any of these possibilities. The third possibility seems unlikely because there was no significant vitamin E effect on muscle iron (Fig. 12).

FIG. 8. Hemoglobin concentrations and transferrin saturation in pigs during the feed trial. Values are means $\pm$ SE. Significant contrasts: control versus the average for all + Fe animals ($p=0.000$).

Moderate elevations in iron stores had a marked effect on oxidative stress, especially as indicated by liver TBARS. This is consistent with reports in the literature. Supplemental vitamin E and to a lesser extent vitamin C protected against this oxidative stress.

Elevated iron stores had a modest effect on the stability of pork, as indicated by TBARS in cooked stored pork and color in raw pork. This agrees with our earlier findings.

FIG. 9. Concentrations (wet weight basis) of total and non-heme iron in fresh livers taken at time of slaughter. Values are means $\pm$ SE. Significant contrasts: control versus the average for all + Fe animals ($p=0.000$ for total and non-heme iron); vitamin E effect ($p=0.000$ for total iron; $p=0.017$ for non-heme iron).

FIG. 10. Lipid peroxidation in fresh livers taken at time of slaughter. Values are means $\pm$ SE. Significant contrasts: control versus the average for all + Fe animals ($p=0.003$); vitamin C effect ($p=0.038$); vitamin E effect ($p=0.000$).

FIG. 11. Red blood cell hemolysis during the feeding trial. Values are means $\pm$ SE. Significant contrasts: control versus the average for all + Fe animals ($p=0.015$); vitamin E effect ($p=0.047$).

FIG. 12. Lipid peroxidation (TBARS) and non-heme iron concentrations in cooked ground pork stores at 4°C in the dark. Values are means $\pm$ SE. Significant contrasts: vitamin E effect on TBARS slopes for days 0-4 ($p=0.042$); vitamin E effect on TBARS individual grand means for days 0-4 ($p=0.005$); control versus average slopes and individual grand means for non-heme iron for days 0-10 (0.039).

The vitamin E data are summarized in Tables 12a and 12b. Iron loading appeared to reduce vitamin E levels slightly, and especially in muscles. Vitamin E supplementation dramatically increased tissue vitamin E levels. Vitamin C supplementation had a protective effect on vitamin E, especially in vitamin E-supplemented animals. Cooking caused dramatic reduction in vitamin E levels, and storage results in still further losses.

TABLE 12A. Vitamin E in raw tissues from freshly slaughtered pigs.
Values are μg α -tocopherol/g tissue.

Treatment	Liver	Leaf Fat	Ground Ham
Control	1.83	1.63	5.50
Fe-only	1.63	1.23	3.77
Fe + C	1.96	0.93	3.77
Fe + E	12.72	2.42	9.75
Fe + E + C	13.84	2.36	14.47

TABLE 12B. Vitamin E in cooked stored ground ham. Meat was cooked and stored at 4°C. Values are μg α -tocopherol/g tissue.

Treatment	Length of storage in days				
	0	2	4	6	8
Control	0.31	0.24	0.08	0.05	0.00
Fe-only	0.32	0.16	0.02	0.00	0.00
Fe + C	0.36	0.23	0.14	0.00	0.00
Fe + E	1.02	1.19	0.57	0.00	0.15
Fe + E + C	1.69	1.19	0.79	0.00	0.14

GROWTH AND HEMATOLOGY - TURKEY

The effect of age and the supplementation of Fe unsupplemented diet with three levels of Fe (0, 25 and 100 mg/kg) on growth and blood hemoglobin level was evaluated. Birds were bled and slaughtered at 45, 75 and 157 days of age. Results are summarized in Table 13. Neither of the treatments significantly affected body weight and blood hemoglobin levels. However, the latter increased by age. Feed efficiency was also not affected by the treatments in this experiment, as well as in the other ones. It was found that Fe unsupplemented diet contained 130 ± 7 mg iron/kg.

TABLE 13. Effect of various levels of Fe supplementation on body weight and blood hemoglobin of turkeys at various ages (Experiment 1)¹

	Fe supplement (mg/kg diet) ²			SEM	P<
	0	25	100		
Body weight (g)					
45 d	2494	2489	2491	112.9	NS
75 d	6000	6137	6500	308.9	NS
157 d	16701	16422	17060	335.4	NS
Hemoglobin (g/dl)					
45 d	9.0	9.5	9.6	0.267	NS
75 d	9.9	10.0	9.4	0.176	NS
157 d	12.6	11.9	12.1	0.366	NS

¹Means of 5 turkeys. No significant ($P>0.05$) differences, in both variables, were observed due to the Fe supplementation.

²The basal diet did not contain Fe supplement.

Data on BW, liver weight and the concentration of hemoglobin, calcium and phosphorous in the blood at 70 and 162 d of age are summarized in Tables 14 and 15. The treatments did not affect body and liver weight at either age (only values of the two extreme treatments are determined at 70 d of age). Blood hemoglobin at both ages were significantly lower in birds fed the Fe-unsupplemented diet than in those fed the diet supplemented with 250 or 500 mg Fe/kg (factorial effect at 162 d of age). Intermediate values were observed in birds fed the diet supplemented with 100 mg Fe/kg. Blood hemoglobin concentrations were increased by age in birds of the two treatments, evaluated at both age periods (birds fed the Fe-unsupplemented diet and those fed the diet supplemented with 500 mg Fe/kg). The treatments did not affect plasma calcium and phosphorous levels at 70 d of age, but at 162 d of age, turkeys fed the diets containing 250 and 500 mg Fe/kg had significantly higher plasma calcium than those fed the Fe-unsupplemented diet and the diet supplemented with 100 mg Fe/kg (factorial effect). Weight loss of meat during the 135 d of storage was not affected by dietary treatments (average 1.1%, data not detailed).

TABLE 14. Effect of high level of dietary Fe supplement on body weight and the concentrations of hemoglobin, calcium and phosphorus in the blood of turkeys at 70 days of age (Experiment 2)¹

	Fe supplement (mg/kg diet) ²		SEM	P<
	0	500		
Body wt (g)	5680	5678	108.3	NS
Liver wt (g)	72.5	73.3	2.11	NS
Liver wt (% BW)	1.3	1.3	0.035	NS
Blood				
Hemoglobin (g/dl)	8.9 ^b	10.1 ^a	0.25	0.01
Calcium (mg/dl)	10.6	10.7	0.18	NS
Phosphorus (mg/dl)	6.7	6.4	0.41	NS

¹Means of 5 turkeys.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix and 28 mg vitamin E/kg supplemented by the vitamin mix.

³Fe was supplemented at a level of 250 mg/kg diet up to 4 weeks of age.

TABLE 15. Effect of various levels of Fe (Fe sup.) and vitamin E (Vit. E) on hemoglobin, calcium and phosphorus in the blood of turkeys at 162 days of age (Experiment 2)¹

Dietary variables ²		Body wt.	Liver wt.	Blood		
Fe sup.	Vit. E			Hemoglobin	Calcium	Phosphorus
(mg/kg)		(g)	(% BW)	(g/dl)	(mg/dl)	
0	0	16,760	0.67	13.7 ^b	12.0 ^{ab}	6.1
0	28	17,457	0.62	13.8 ^b	10.9 ^c	6.0
100	0	17,627	0.64	14.4 ^b	11.2 ^{bc}	5.6
100	28	17,180	0.66	14.6 ^{ab}	11.7 ^{abc}	6.5
250	0	17,202	0.62	15.7 ^a	12.0 ^{ab}	6.3
250	28	16,206	0.65	15.5 ^{ab}	12.5 ^a	5.9
500	0	15,752	0.72	15.2 ^{ab}	12.4 ^a	6.0
500	28	16,962	0.71	14.4 ^b	12.1 ^a	6.6
SEM		484	0.037	0.42	0.27	0.31
Factorial effects						
Fe	0	17,108	0.64	13.7 ^c	11.4 ^b	6.0
	100	17,403	0.65	14.5 ^{bc}	11.4 ^b	6.0
	250	16,704	0.63	15.6 ^a	12.2 ^a	6.1
	500	16,357	0.71	14.8 ^{ab}	12.2 ^a	6.3
P<		NS	NS	0.01	0.01	NS
Vit. E	0	16,835	0.66	14.7	11.9	6.0
	28	16,951	0.66	14.5	11.8	6.2
P<		NS	NS	NS	NS	NS
Fe x Vit E						
P<		NS	NS	NS	0.05	NS

^{a,b,c}Values not followed by the same letter differ significantly ($P < 0.05$).

¹Means of 5 turkeys

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

Data on BW, liver wt. and the concentration of hemoglobin the blood at 91 and 158 d of age from experiment 3 are summarized in Table 16. BW was slightly, but significantly affected by the treatments at 91, the highest value was observed in birds fed the diet supplemented with 250 mg Fe/kg without vitamin E, and the lowest value with those fed the diet supplemented with 150 mg Cu/kg without vitamin E. On a factorial basis the difference between birds fed on the diet supplemented with 250 mg Fe/kg and the diet supplemented with 150 mg Cu/kg was significant,

while vitamin E did not affect this variable. Neither of the treatments nor the factors affected BW at 158 d of age. Blood hemoglobin levels were not affected at both ages, by any of the dietary supplements, but levels observed at older age were higher than in younger birds, in agreement with the previous experiments. Factorial ANOVA with data of both age periods, not detailed here for the sake of brevity, demonstrate that blood hemoglobin levels were affected significantly ($P < 0.001$) only by age.

TABLE 16. Effect of various levels of dietary Fe (Fe sup.), copper (Cu sup.) and vitamin E (Vit. E) on body weight and the concentration of hemoglobin in the blood of turkeys at 91 and 158 d of age (Experiment 3)¹

Dietary variables ²			Age (d)				
Fe sup.	Cu sup.	Vit E	91		158		
(mg/kg)			Body wt (g)	Hemoglobin (g/dl)	Body wt (g)	Liver wt (% BW)	Hemoglobin (g/dl)
0	0	0	7541 ^{bc}	10.8	14857	0.82	13.5
0	0	28	8162 ^{ab}	10.4	15445	0.78	14.1
250	0	0	8243 ^a	11.1	16298	0.85	14.3
250	0	28	8210 ^a	10.7	15965	0.89	14.1
500	0	0	8136 ^{abc}	11.2	15314	0.79	13.8
500	0	28	7686 ^{abc}	11.0	15438	0.86	13.7
0	150	0	7467 ^c	11.0	15006	0.83	13.9
0	150	28	7648 ^{abc}	10.9	16390	0.79	13.5
SEM			218	0.36	612	0.08	0.70
Factorial effects							
Fe sup.	0		7851 ^{ab}	10.6	15151	0.80	13.8
	250		8226 ^a	10.9	16131	0.87	14.2
	500		7911 ^{ab}	11.1	15376	0.82	13.7
Cu sup.	150		7557 ^b	10.9	15698	0.82	13.7
	P<		0.05	NS	NS	NS	NS
Vit E	0		7847	11.0	15369	0.82	13.9
	28		7926	10.7	15809	0.83	13.8
	P<		NS	NS	NS	NS	NS
Fe (Cu) x Vit							
	E						
	P<		NS	NS	NS	NS	NS

^{abc}Values not followed by the same letter differ significantly ($P < 0.05$).

¹Means of 5 turkeys.

²The basal diet contained 20 mg Fe/kg and 5 mg Cu/kg, supplemented by the mineral mix, but not vitamin E supplement.

Body weight and relative liver weight at 112 d of age, as well as blood hemoglobin concentrations at 84 and 112 d of age were not affected significantly by the treatments, as well as by the factors (Table 17) (experiment 4). Blood hemoglobin levels were increased by age. Upon dissection, we noticed that the color of the Fe-dextran injected drumstick was brown, as the color of the Fe-dextran. The color of the thigh (of the same side) was normal.

TABLE 17. Effect of dietary vitamin E (Vit. E) level and Fe-dextran injections on body and liver weights and blood hemoglobin of turkeys at 112 d of age (Experiment 4)¹

Treatment ²		Body wt.	Wt. gain (84-112 d)	Liver	Hemoglobin at age (d)	
Dietary Vit. E	Fe-dextran				84	112
(mg/kg)		(g)	(g)	(% BW)	(g/dl)	
0	No	9988	2574	1.14	10.1	11.2
0	Yes	10600	3208	1.27	10.9	12.5
150	No	10715	3470	0.98	10.6	12.1
150	Yes	10798	3601	1.13	10.7	12.2
SEM		528	345	0.092	0.49	0.44
Factorial effects						
Vit E	0	10294	2891	1.20	10.5	11.8
	150	10756	3535	1.06	10.6	12.1
P<		NS	NS	NS	NS	NS
Fe-dextran	No	10351	3022	1.06	10.3	11.6
	Yes	10699	3404	1.20	10.8	12.3
P<		NS	NS	NS	NS	NS

¹Means of 4, 8 and 8 turkeys per treatment, vitamin E level and Fe-dextran injection, respectively. Body weight at 56 and 84 d of age averaged 3446 and 7311 g, respectively. At age 84 d this variable was not affected by dietary vitamin E level.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement. Vitamin E treatments were given from 56 to 112 d of age and Fe-dextran injections (to the left drumstick) from 84 to 105 d of age.

BW and relative liver weight at 104 d of age, as well as blood hemoglobin concentration at 84 and 154 d of age, were not affected significantly by the various treatments (data not shown).

Chemical data

The effect of age and the supplementation of Fe unsupplemented diet with three levels of Fe (0, 25 and 100 mg/kg) on the accumulation of total and "free" iron in the drumstick muscle was evaluated and presented in Table 18. The results show no differences between the treatments concerning total iron and a trend (but not significantly) to decrease free iron at old age.

Data (from Experiment 2 - turkeys) on the effect of the treatments on meat stability at 4°C of fresh drumsticks (Fig. 13) and frozen thigh (Table 19) are presented.

The results demonstrated the addition of α -tocopherol significantly improved the stability of the muscle stored at 4°C (Fig. 13). This variable was not markedly affected by the various levels of Fe supplementation. Data observed with muscle stored at -18°C show that supplementation of the diets with Fe decreased, at times significantly (on a factorial basis), the TBARS value (initial and after incubation) determined after 25 d of storage (Table 19). This effect of Fe was proportional to its level of supplementation. However, after additional 95 d of storage, the protective effect of Fe disappeared. Vitamin E supplementation significantly prevented the increase in initial TBARS values (on a factorial basis) after 25 d of storage. Otherwise, it had no effect on meat stability. Initial

TBARS values markedly increased during the additional 95 of storage at -18°C .

TABLE 18. Effect of various levels of Fe supplementation on total iron and chelatable iron ("free" iron) in drumstick muscle (Experiment 1).

	Fe supplement (mg/kg diet) ²			SEM	P<
	0	25	100		
Total iron (µg/g)					
45 d	12.8	12.8	12.4	0.7	NS
75 d	11.3	11.4	12.1	0.6	NS
157 d	14.5	14.7	14.8	1.3	NS
Free iron (µg/g)					
45 d	1.16	1.14	1.16	0.09	NS
75 d	0.62	0.50	0.54	0.10	NS
157 d	0.80	0.83	0.90	0.15	NS

¹Means of 5 turkeys. No significant ($P>0.05$) differences in both variables were observed due to the Fe supplementation.

²The basal diet did not contain Fe supplement, however it did contain ~120 mg iron, from the natural feed components.

Data (from Experiment 3 - turkeys) on the stability of turkey meat is presented in Figure 14 for fresh muscles at 4°C and in Table 20 for frozen muscles.

The results on the storage of fresh muscles at 4°C show that increasing dietary Fe level markedly decreased meat stability, particularly with the vitamin E unsupplemented diets. Vitamin E *per se* improved stability. The results on stability of the thigh muscle stored at -18°C (Table 20) did not demonstrate a significant effect, either to Fe level or to vitamin E supplementation. It seems that freezing and thawing increases the rate of oxidation and by this prevents small differences between treatments, as

was obtained by storage of the muscle at 4°C. Increasing dietary Cu, from 5 to 155 mg/kg, did not affect the stability of meat stored at -18°C (Table 20).

FIG. 13. The effect of various levels of dietary Fe and vitamin E (28 mg/kg diet) on the stability of turkey drumsticks during storage at 4°C (Experiment 2).

TABLE 19. Effect of various levels of dietary Fe (Fe sup.) and vitamin E (Vit. E) on the stability of thigh meat during storage (Experiment 2)¹

Dietary variables ²		Storage duration (d) ³			
Fe sup.	Vit. E	25		120	
		Initial level	After incubation ⁴	Initial level	After incubation
(mg/kg)		TBARS values ⁵			
0	0	2.97 ^a	28.6 ^a	6.38	31.6
0	28	1.83 ^{ab}	30.4 ^a	6.78	32.7
100	0	2.16 ^{ab}	26.9 ^{ab}	4.86	28.1
100	28	1.67 ^{ab}	26.8 ^{ab}	6.00	36.0
250	0	2.16 ^{ab}	23.7 ^{ab}	6.24	32.6
250	28	0.83 ^b	18.1 ^b	5.22	29.9
500	0	1.32 ^{ab}	17.7 ^b	5.92	30.2
500	28	0.57 ^b	17.3 ^b	6.20	29.0
SEM		0.58	3.37	0.866	2.32
Factorial effects					
Fe	0	2.40	29.5 ^a	6.58	32.1
	100	1.91	26.9 ^{ab}	5.43	32.0
	250	1.49	20.9 ^{bc}	5.73	31.2
	500	0.94	17.5 ^c	6.06	29.6
P<		0.10	0.01	NS	NS
Vit. E.	0	2.15	24.2	5.85	30.6
	28	1.22	23.1	6.05	31.9
P<		0.05	NS	NS	NS
Fe x Vit. E					
P<		NS	NS	NS	NS

^{a,b,c} Values not followed by the same letter differ significantly (P<0.05).

¹ Means of 5 turkeys

² The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

³ At -18°C

⁴ In water bath at 37°C for 1 h under constant shaking

⁵ Expressed as milligrams sodium salt of malonaldehyde-bis-bisulfite per kilogram meat.

FIG. 14. The effect of various levels of dietary Fe and vitamin E (28 mg/kg diet) on the stability of turkey drumsticks during storage at 4°C (Experiment 3).

TABLE 20. Effect of various levels of dietary Fe (Fe sup.), copper (Cu sup.) and vitamin E (Vit. E) on the stability of thigh meat during storage (Experiment 3)²

Dietary variables ²			Storage duration (d) ³			
			25		120	
Fe sup.	Cu sup.	Vit. E	Initial level	After incubation ⁴	Initial level	After incubation ⁵
(mg/kg)			TBARS values ⁶			
0	0	0	1.6	29.0	2.4	18.5
0	0	28	1.1	29.8	1.5	18.6
250	0	0	1.5	32.6	3.0	19.0
250	0	28	1.2	31.0	2.3	16.6
500	0	0	1.8	33.5	2.7	19.8
500	0	28	1.3	30.8	2.8	15.9
0	150	0	1.4	33.8	3.0	20.9
0	150	28	0.39	33.3	2.4	21.7
SEM				2.37	1.01	2.86
Factorial effects						
Fe		0	1.35	29.4	1.95	18.6
		250	1.35	31.8	2.65	17.8
		500	1.55	32.1	2.75	17.8
Cu		150	1.40	33.5	2.70	21.3
P<			NS	NS	NS	NS
Vit. E.		0	1.56	32.3	2.78	19.5
		28	1.25	31.2	2.25	18.2
P<			NS	NS	NS	NS
Fe (Cu) x Vit. E						
P<			NS	NS	NS	NS

¹Means of 5 turkeys

²The basal diet contained 20 mg Fe/kg and 5 mg Cu/kg, respectively, supplemented by the mineral mix, but not vitamin E supplement.

³At -18°C

^{4,5}In water bath at 37°C for 1 h and 1/2 h, respectively, under constant shaking.

⁶Expressed as milligrams sodium salt of malonaldehyde-bis-bisulfite per kilogram meat.

Fe overload by injection of Fe-dextran (turkey)

Since we were not able to increase the concentrations of iron in the muscle by increasing its level in the diets, we decided to introduce iron directly into the muscle turkeys' blood by injection of Fe-dextran (used to prevent anemia in swine), in order to evaluate better the effects of overload iron on muscle lipid peroxidation (Experiment 4).

The results presented (Table 21) show that turkeys injected with Fe-dextran accumulate significantly more iron in the liver. However, the accumulation of iron in the right leg muscles are very small compared with the saline-injected control, and do not differ significantly. These data demonstrated that the turkey liver could accumulate very high concentrations of iron, and that the transfer of the iron from the liver to muscles is regulated by specific mechanisms (Theil and Aisen, 1987).

TABLE 21. Effect of dietary Vitamin E (Vit. E) level and Fe-dextran injections on total iron content of the right and left drumsticks and liver of turkeys (Experiment 4).

Treatment ²		Fe-Total ¹		
Dietary Vit. E	Fe-	Right-D	Left-D	Liver
(mg/kg)	dextran	µg/g wet wt.		
0	No	14.6	-	94.4
0	Yes	15.2	25.9	126.9
150	No	13.1	-	105.3
150	Yes	15.4	24.8	126.6
SEM		2.6	3.2	7.7
Factorial effects				
Vit. E	0	14.9	25.9	110.6
	150	14.2	24.8	116.2
P<		NS	NS	NS
Fe-dextran	No	13.8	13.8	100.1
	Yes	15.3	25.3	126.7
P<		NS	NS	NS

¹Means of 4, 8 and 8 turkeys per treatment, vitamin E level and Fe-dextran injection, respectively. Vitamin E treatments were given from 56-112 d of age, and Fe-dextran injections (to the left drumstick) from 84-105 d of age.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

TABLE 22. Effect of dietary Vitamin E (Vit. E) level and Fe-dextran injections on "free" iron content of the right and left drumsticks of (injected) turkeys (Experiment 4)

Treatment		Fe-"Free"	
Dietary Vit. E	Fe-dextran	Right-D	Left-D
(mg/kg)		$\mu\text{g/g wet wt.}$	
0	No	1.20	-
0	Yes	1.30	7.21
150	No	0.80	-
150	Yes	0.90	4.29
SEM		0.20	1.50
Factorial effects			
Vit. E	0	1.25	7.21
	150	0.85	4.29
P<		0.05	0.05
Fe-dextran	No	1.00	1.00
	Yes	1.05	6.75
P<		NS	0.01

TABLE 23. Effect of dietary Vitamin E (Vit. E) level and Fe-dextran injections on α -tocopherol content of the right and left drumsticks and liver of turkeys (Experiment 4)

Treatment		α -tocopherol		
Dietary Vit. E	Fe-dextran	Right-D	Left-D	Liver
(mg/kg)		$\mu\text{g/g wet wt.}$		
0	No	3.3 ^a	-	2.1 ^a
0	Yes	4.3 ^a	1.8 ^a	2.8 ^a
150	No	15.4 ^c	-	11.1 ^b
150	Yes	11.9 ^b	6.5 ^b	10.0 ^b
SEM		1.4	1.8	1.2
Factorial effects				
Vit. E	0	3.8	1.8	2.4
	150	13.6	6.5	11.5
P<		0.01	0.01	0.01
Fe-dextran	No	7.9	7.9	6.6
	Yes	8.1	4.1	6.4
P<		NS	0.05	NS

^{a,b,c}Values not followed by the same letter differ significantly ($P < 0.05$).

The concentration of total and "free" iron of the right muscle of the Fe-dextran-injected birds did not differ from values observed in saline-injected birds. However, the left drumstick (the injected site) contained significantly higher amounts of total and "free" iron (Tables 21, 22). Muscles of all turkeys fed the high (150 mg/kg) vitamin E diet contained significantly lower amounts of "free" iron than muscles of birds fed the low vitamin E diet. It seems that lipid peroxidation is affected by free iron, but also peroxidative reactions affected the release of iron from iron-bound stores (Table 23 and Fig. 15).

Vitamin E supplementation significantly improved the stability of the fresh muscle stored at 4°C (Fig. 15) or at -18°C (Tables 24, 25). The injection of Fe-dextran to the left drumstick decreased, but not significantly, the stability of the muscle at 4°C (Fig. 15) or at -18°C (Table 24) from the right drumstick and the thigh. However this injection significantly decreased the stability of the muscle from the left drumstick (Fig. 15) or thigh (Table 25).

The effect of Fe-dextran was not significant after 108 d of storage at -18°C. It is worth noting that the initial TBARS value in the rights muscles at time 0 at 4°C, or after 15 d at -18°C of storage were significantly affected by the interaction between vitamin E and Fe-dextran (Fig. 15 and Table 25). That is because the Fe-dextran decreased stability only in birds fed the low vitamin E diet. Initial TBARS values were markedly increased during storage at 4°C or -18°C. Dramatic inhibition of the accumulation of TBARS was demonstrated in samples from the Fe-dextran injected drumstick on vitamin E diet (Fig. 15).

FIG. 15. The effect of iron (Fe-dextran) injections to the left drumstick of turkeys fed two levels of vitamin E (0 and 150 mg/kg diet) on the muscle stability at 4°C (Experiment 4).

L=left drumstick; R=right drumstick

Vitamin E, 0 or 150 mg/kg diet

TABLE 24. Effect of dietary Vitamin E (Vit. E) level and Fe-dextran injections on the stability of meat from the right thigh (the non-injected side) during storage (Experiment 4)¹

Treatment ²		Storage duration (d) ³				
		15		30		108
Dietary Vit. E	Fe-dextran	Initial level	After incubation ⁴	Initial level	After incubation	Initial level
(mg/kg)		TBARS values ⁵				
0	No	1.08 ^a	18.0 ^a	2.20 ^a	20.3 ^{ab}	5.21 ^a
0	Yes	0.41 ^b	18.9 ^a	2.17 ^a	22.8 ^a	4.56 ^{ab}
150	No	0.06 ^b	6.0 ^b	0.14 ^b	5.0 ^c	2.58 ^b
150	Yes	0.16 ^b	11.7 ^{ab}	0.37 ^b	12.4 ^{bc}	2.88 ^{ab}
SEM		0.159	2.81	0.389	2.62	0.758
Factorial effects						
Vit. E	0	0.74	18.4	2.18	21.6	4.88
	150	0.11	8.8	0.26	8.7	2.73
P<		0.01	0.01	0.001	0.001	0.05
Fe-dextran	No	0.57	12.0	1.17	12.6	3.89
	Yes	0.28	15.3	1.27	17.6	3.72
P<		NS	NS	NS	NS	NS
Vit. E x Fe-dextran						
P<		0.05	NS	NS	NS	NS

^{a,b,c}Values not followed by the same letter differ significantly ($P < 0.05$).

¹Means of 4, 8 and 8 turkeys per treatment, vitamin E level and Fe-dextran injection, respectively.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement. Vitamin E treatments were given from 56 to 112 d of age and Fe-dextran injections (to the left drumstick) from 84 to 105 d of age.

³At -18°C.

⁴In water bath at 37°C for 1 h under constant shaking.

⁵Expressed as milligrams sodium salt of malonaldehyde-bis-bisulfite per kilogram meat.

TABLE 25. Effect of dietary Vitamin E (Vit. E) level and Fe-dextran injections on the stability of meat from the left thigh (the injected side) during storage (Experiment 4)¹

Treatment ²		Storage duration (d) ³				
Dietary Vit. E (mg/kg)	Fe-dextran	23		36		107
		Initial level	After incubation ⁴	Initial level	After incubation	Initial level
TBARS values ⁵						
0	No	0.53 ^b	14.1 ^{bc}	1.15 ^b	15.7 ^b	6.55 ^{ab}
0	Yes	2.10 ^a	22.9 ^a	4.34 ^a	28.4 ^a	9.77 ^a
150	No	0.30 ^b	5.9 ^c	0.24 ^b	6.7 ^b	5.38 ^{ab}
150	Yes	0.97 ^{ab}	16.9 ^{ab}	1.66 ^{ab}	17.3 ^b	4.07 ^b
SEM		0.46	2.76	0.99	3.38	1.45
Factorial effects						
Vit. E	0	1.31	18.5	2.74	22.0	8.16
	150	0.63	11.4	0.95	12.0	4.72
P<		NS	0.05	NS	0.05	0.05
Fe-dextran	No	0.41	10.0	0.69	11.2	5.96
	Yes	1.53	19.9	3.00	22.8	6.92
P<		0.05	0.01	0.05	0.01	NS
Vit. E x Fe-dextran						
P<		NS	NS	NS	NS	NS

^{a,b,c} Values not followed by the same letter differ significantly ($P < 0.05$).

¹ Means of 4, 8 and 8 turkeys per treatment, vitamin E level and Fe-dextran injection, respectively.

² The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement. Vitamin E treatments were given from 56 to 112 d of age and Fe-dextran injections (to the left drumstick) from 84 to 105 d of age.

³ At -18°C.

⁴ In water bath at 37°C for 1 h under constant shaking.

⁵ Expressed as milligrams sodium salt of malonaldehyde-bis-bisulfite per kilogram meat.

TABLE 26. Effect of two levels of dietary Fe (Fe sup.) and three levels of Vitamin E (Vit. E) on the content of total iron and "free" iron (Experiment 5)¹

Dietary variables ²		Fe "free"	Fe-total	
Fe sup.	Vit. E	Drumstick	Drumstick	Liver
(mg/kg)		(µg/g wet wt.)		
0	0	0.79	13.5	106.5
0	28	0.81	13.1	104.3
0	150	0.78	14.0	99.1
500	0	1.40	14.3	117.8
500	28	0.84	13.7	119.1
500	150	0.82	15.0	130.3
SEM		0.25	2.2	6.5
Factorial effects				
Fe	0	0.79	13.5	103.3
	500	1.02	14.3	122.4
P<		NS	NS	0.05
Vit. E	0	1.09	13.9	112.1
	28	0.82	13.4	109.2
	150	0.80	14.5	114.7
P<		NS	NS	NS

¹Means of 5, 10 and 15 turkeys per treatment, vitamin E level and Fe-supplementation, respectively.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

Combined effects of two levels of dietary Fe and three levels of Vitamin E on the accumulation of those compounds in muscle tissues and liver, and the stability of the meat at 4° and -18°C were studied in experiment 5 - turkeys.

The results show that high Fe-supplementation increased significantly the amount of total iron in the liver, however the amount of total iron and "free" iron in the drumstick does not differ from the control (Table 26).

Vitamin E supplementation increased the amount of α -tocopherol in liver and drumstick tissues, especially with supplementation of 150 mg/kg (Table 27).

TABLE 27. Combined effect of two levels of dietary Fe (Fe sup.) and three levels of Vitamin E (Vit. E) on the content of α -tocopherol in the drumstick and liver of turkeys (Experiment 5)¹

Dietary variables ²		Storage duration (days)		
		0	10	0
Fe sup.	Vit. E	Drumstick		Liver
(mg/kg)		(μ g/g wet wt.)		
0	0	2.48 ^a	1.85 ^a	2.75 ^a
0	28	4.23 ^{ab}	3.58 ^{ab}	4.07 ^{ab}
0	150	10.08 ^c	8.94 ^c	9.31 ^c
500	0	2.92 ^a	2.02 ^a	2.91 ^a
500	28	4.31 ^{ab}	3.34 ^{ab}	3.13 ^{ab}
500	150	10.20 ^c	6.04 ^c	7.58 ^c
SEM		1.20	1.40	1.8
Factorial effects				
Fe sup.	0	5.59	4.79	5.37
	500	5.81	3.80	4.54
P<		NS	NS	NS
Vit. E	0	2.7 ^a	1.93 ^a	2.83 ^a
	28	4.3 ^{ab}	3.46 ^{ab}	3.60 ^{ab}
	150	10.1 ^c	7.49 ^c	8.44 ^c
P<		0.01	0.01	0.01

^{a,b,c}Values not followed by the same letter differ significantly ($P < 0.05$).

¹Means of 5, 10 and 15 turkeys per treatment, vitamin E level and Fe-supplementation, respectively.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

The effect of the lower level of vitamin E (28 mg/kg) on improving meat stability was significant on muscles stored at 4°C, but not at -18°C (Fig. 16 and Table 28, respectively). The treatment with 150 mg/kg vitamin E increased significantly the stability of the meat of both muscles stored at

4°C or -18°C. One should know that freezing, and especially thawing, considerably affect the rate of lipid peroxidation, and this could be the reason why vitamin E, at a level of 28 mg/kg diet, improved the stability of the meat stored at 4°C and not at -18°C.

It is worth noting that there were significant differences between the effects of 28 or 150 mg vitamin E/kg on meat stability only with the low dietary Fe supplementation during storage of the muscle at 4°C, but not with muscles stored at -18°C (Fig. 16, Table 28). Meat stability was consistently unaffected by the supplementation of the diet with 500 mg Fe/kg. The exception was the increased meat stability of the thigh muscle of birds fed the basal diet supplemented with 28 mg vitamin E stored at 4°C, due to the supplementation of this diet with 500 mg Fe/kg.

FIG. 16. Combined effect of two levels of dietary Fe and three levels of vitamin E on the stability of drumstick meat stored at 4°C (Experiment 5).

(Fe mg/kg; Vit. E mg/kg) 0-Fe + 0-Vit. E, ○; 500 Fe + 0-Vit. E, ○; 0-Fe + 28-Vit. E, Δ; 500 Fe + 28 Vit. E, Δ; 0-Fe + 150 Vit. E, □; 500-Fe + 150-Vit. E, □.

TABLE 28. Combined effect of two levels of dietary Fe (Fe sup.) and three levels of vitamin E (Vit. E) on the stability of thigh meat during storage (Experiment 5)¹

Dietary variables ²		Storage duration (d) ³		
Fe sup.	Vit. E	28		101
		Initial level	After incubation ⁴	Initial level
(mg/kg)		TBARS values ⁵		
0	0	2.28 ^a	18.4	6.63 ^a
0	28	1.17 ^b	17.1	4.92 ^{ab}
0	150	1.31 ^{ab}	12.5	4.72 ^{ab}
500	0	1.88 ^{ab}	17.7	5.18 ^{ab}
500	28	1.68 ^{ab}	15.4	4.71 ^{ab}
500	150	1.13 ^b	13.2	2.77 ^b
SEM		0.38	2.38	1.06
Factorial effects				
Fe sup.	0	1.59	16.0	5.42
	500	1.56	15.4	4.22
P<		NS	NS	NS
Vit. E	0	2.08 ^a	18.1 ^a	5.90
	28	1.42 ^{ab}	16.2 ^{ab}	4.81
	150	1.22 ^b	12.8 ^b	3.74
P<		0.05	0.05	NS
Fe x Vit. E				
P<		NS	NS	NS

^{a,b,c}Values not followed by the same letter differ significantly ($P < 0.05$).

¹Means of 5, 10 and 15 turkeys per treatment, vitamin E level and Fe-supplementation, respectively.

²The basal diet contained 20 mg Fe/kg supplemented by the mineral mix, but not vitamin E supplement.

³At -18°C.

⁴In water bath at 37°C for 1 h under constant shaking.

⁵Expressed as milligrams sodium salt of malonaldehyde-bis-bisulfite per kilogram meat.

FIG. 17. Correlation between muscle lipid peroxidation in stored muscles at 4°C (after 10 days), as affected by the amount of *in situ* α -tocopherol content of muscle tissues (Experiment 5). Unsupplemented Fe, O; 500 mg Fe/kg diet, $\square$.

Lipid peroxidation was inhibited significantly in turkey muscles contained at least $\sim 10 \mu\text{g/g}$ α -tocopherol wet wt. (Fig. 17). Although the muscle also contained β and γ -tocopherols (results not shown), only α -tocopherol concentration decreased during storage, promoting an antioxidative effect.

THE EFFECT OF DIETARY IRON LEVEL ON CHICKS MUSCLE LIPID PEROXIDATION AND *IN VIVO* STATUS OF α -TOCOPHEROL

Growth, hematology and calcium status (chicks)

The regular feeds for turkeys and chickens without supplementation of iron contained "naturally" ~120 mg iron. During our recent work, it was not possible to decrease the amount of this iron in the regular diet.

A synthetic feed (Table 2) was prepared to search the effect of Fe on the growth and muscle stability of chicks.

Experiment 1 (chicks). The effect of supplementation of a Fe-deficient diet with three concentrations of Fe (0, 25 and 150 mg/kg) on the development, blood hemoglobin and meat stability was evaluated. Cholecalciferol was added to the experimental diets at a concentration of 37.5 μ g/kg. The starter experimental diets which had been fed up to 13 d of age contained, by mistake, only 0.533% added arginine and their lysine:arginine ratio (1.43:1) was wider than required for optimal development. This error was then corrected by adding 0.647% arginine, so that the above mentioned ratio was about 1:1. The experiment was terminated at 52 d of age.

Body weights during the course of Experiment 1 are detailed in Table 29. Significant differences between chicks fed the diets supplemented with 0 and 150 mg Fe/kg were already observed at the age of 6 d, and between the formers and those fed the diet supplemented with 25 mg Fe/kg at the age of 20 d. At that age, the difference between chicks fed on the commercial diet and those fed on the diet with the highest Fe supplement

became significant. Body weights of chicks fed the diets supplemented with 25 and 150 mg Fe/kg were essentially the same throughout the experiment. Leg weakness was noticed in a few chicks fed on the three experimental diets based on the synthetic diet at the age of 12 d. The shanks of the chicks fed on these diets were thinner than normal, and their feathers had an unusual frizzly appearance. These phenomena partly disappeared after the arginine content of these diets was increased at the age of 13 d (see above).

TABLE 29. Effect of Fe supplements to Fe-deficient diet on body weight (g) during the course of Experiment 1^{1,2}

Age (d)	Basal diet				SEM	P<
	Fe-deficient			CD ³		
	Fe supplementation (mg/kg)					
	0	25	150			
6	117 ^b	118 ^b	132 ^a	126 ^{ab}	3.4	0.05
13	191 ^c	223 ^{bc}	253 ^{ab}	295 ^a	15.5	0.001
20	334 ^d	453 ^c	535 ^b	614 ^a	25.6	0.001
27	521 ^c	743 ^b	844 ^b	1047 ^a	34.3	0.001
41	812 ^c	1446 ^b	1315 ^b	2069 ^a	50.2	0.001
52	1087 ^c	1841 ^b	1705 ^b	2931 ^a	86.5	0.001

^{a,b,c} Within rows, values not followed by the same superscript differ significantly (P<0.05).

¹ Average of 6 chicks.

² Body wt. at 1 d of age average 41 g

³ Commercial diet.

Data observed at 52 d of age are summarized in Table 30. Hemoglobin concentrations in chicks fed the Fe-deficient diet were significantly lower than in the chicks of the other treatments, whose hemoglobin levels were essentially the same. Relative liver weight was significantly higher in chicks fed the Fe-deficient diet than in chicks of the other treatments.

TABLE 30. Effect of Fe supplements to Fe-deficient diet on body and liver weights, concentration of hemoglobin and calcium in blood and on ossification scores of the sternum of 52 d old broilers (Experiment 1)¹

Variable	Basal diet			CD ²	SEM	P<
	Fe-deficient Fe supplementation (mg/kg)					
	0	25	150			
Body wt ² (g)	1087 ^b	1841 ^b	1705 ^b	2931 ^a	86.5	0.001
Liver wt (g)	28.1 ^b	34.2 ^b	33.1 ^b	48.6 ^a	2.1	0.001
Liver wt (% BW)	2.6 ^a	1.8 ^b	1.9 ^b	1.6 ^b	0.10	0.001
Hemoglobin (g/100 ml blood)	3.5 ^c	8.2 ^{ab}	7.8 ^b	8.6 ^a	0.23	0.001
Calcium mg/dl plasma)	10.7 ^a	8.2 ^b	7.2 ^b	10.6 ^a	0.40	0.001
Ossification scores ³	3.8 ^{ab}	2.7 ^b	2.7 ^b	5.0 ^a	0.53	0.05

^{a,b,c}Within rows, values not followed by the same superscript differ significantly (P<0.05).

¹Average of 6 chicks.

²Commercial diet

³The scores ranged from 1 = soft cartilaginous bone to 5 = hard well ossified bone.

During the dissection it was observed that the sternum bones of almost all the birds which had been fed on the synthetic diets were soft and contained cartilaginous areas. The scoring of the ossification demonstrated a significant difference between chicks fed on diets supplemented with 25 and 150 mg Fe/kg, and those fed on the commercial diet. Intermediate values were observed in chicks fed on the Fe-unsupplemented diet. Calcium concentrations in the plasma were similar in chicks fed on the Fe-unsupplemented and the commercial

diets, and significantly higher than those of the birds fed the Fe-supplemented synthetic diets.

Experiment 2 (chicks). The effect of the supplementation of the Fe-deficient diet containing 75 µg cholecalciferol/kg with two concentrations of Fe (10 and 150 mg/kg) on the development, the concentrations of hemoglobin; calcium and phosphorus in blood; tibia ash; duodenal calbindin; kidney 25-hydroxy cholecalciferol 1-hydroxylase [25(OHD₃-1-hydroxylase] activity and meat stability was determined. The starter diet was fed until the termination of the experiment at 35 d of age.

Body weights of the chicks fed the diets supplemented with either 10 or 150 mg Fe/kg were similar up to 14 d of age, afterwards a significant difference between these treatments was observed (Table 31). Body weights of the chicks fed on the commercial diet were significantly higher than those of the birds fed the diet supplemented with 150 mg Fe/kg, already at 7 d of age.

Data observed at 35 d of age are summarized in Table 32. Hemoglobin concentrations were significantly lower in chicks fed on the diet supplemented with 10 mg Fe/kg than in those fed the diet supplemented with 150 mg Fe/kg and the commercial diet. Values observed in chicks fed on the commercial diet were significantly lower than those determined in chicks fed on the diet supplemented with 150 mg Fe/kg. There was no significant difference in absolute liver weight between the chicks fed on the diet supplemented with 150 mg Fe/kg and those fed on the commercial diet. Relative liver weight was higher, but not significantly, in birds fed on the synthetic diets. The treatments did not affect the level of

plasma calcium, but plasma phosphorus was significantly lower in chicks fed the diet supplemented with 10 mg Fe/kg. There were no significant differences among the treatments in the ash content of the tibia and in duodenal calbindin. The activity of kidney 25(OH)D₃-1-hydroxylase was significantly higher in chicks fed on the diet supplemented with 10 mg Fe/kg than in those fed on the diet supplemented with 150 mg Fe/kg, and significantly higher in the latter than in those fed on the commercial diet.

Experiment 3 (chicks). The combined effect of the supplementation of the Fe-deficient diet with three concentrations of Fe (0, 50 and 150 mg/kg) and two concentrations of cholecalciferol (25 and 62.5 µg/kg) on the development, the concentrations of hemoglobin; calcium and phosphorus in blood; tibia ash; duodenal calbindin; kidney 25(OH)D₃-1-hydroxylase and 25(OH)D₃-1-24-hydroxylase activities and meat stability was determined. The experiment was terminated at 27 d of age.

TABLE 31. Effect of Fe supplements to Fe-deficient diet on body weight (g) during the course of Experiment 2^{1,2}

Age (d)	Basal diet			SEM	P<
	Fe-deficient		CD ³		
	Fe supplementation (mg/kg)				
	10	150			
7	138 ^b	133 ^a	167 ^a	3.4	0.001
14	299 ^b	318 ^b	418 ^a	8.0	0.001
18	434 ^c	487 ^b	623 ^a	10.6	0.001
21	533 ^c	618 ^b	799 ^a	13.4	0.001
28	878 ^c	993 ^b	1304 ^a	27.5	0.001
35	1229 ^c	1419 ^b	1827 ^a	86.5	0.001

^{a,b,c} Within rows, values not followed by the same superscript differ significantly (P<0.05).

¹ Average of 7 chicks.

² Body wt. at 1 d of age average 48 g.

³ Commercial diet.

TABLE 32. Effect of Fe supplements to Fe-deficient diet on body and liver weights, the concentrations of hemoglobin, calcium and phosphorous in the blood, the weight of the tibia and its ash content, duodenal mucosa calbindin (calcium binding protein, CaBP) and the activity of kidney 25(OH)D₃-1-hydroxylase (1-HYD) in 35-d old broiler chicks (Experiment 2)¹

Variable	Basal diet			SEM	P<
	Fe-deficient Fe supplementation (mg/kg)		CD ²		
	10	150			
Body wt ² (g)	1229 ^c	1419 ^b	1827 ^a	46.2	0.001
Liver wt (g)	30.0 ^b	36.3 ^a	40.9 ^a	1.59	0.001
Liver wt (% BW)	2.4 ^a	2.6 ^a	2.2 ^a	0.10	NS
Blood					
Hemoglobin (g/dl)	6.0 ^c	8.8 ^a	7.9 ^b	0.26	0.001
Calcium (mg/dl)	10.2 ^a	10.5 ^a	10.8 ^a	0.35	NS
Phosphorus (mg/dl)	5.5 ^b	7.0 ^a	6.6 ^a	0.31	0.05
Tibia					
Wt (g)	4.26 ^c	5.61 ^b	6.66 ^a	0.17	0.001
Wt (% BW)	0.35 ^b	0.40 ^a	0.36 ^{ab}	0.01	0.05
Ash (g)	1.48 ^c	1.92 ^b	2.47 ^a	0.07	0.001
Ash (%)	34.8	34.1	37.1	0.89	NS
Duodenal					
CaBP (μ/g)	206	458	345	84.4	NS
Kidney					
1-HYD (pmole/g/ 15min)	33.8 ^a	18.9 ^b	4.2 ^c	4.4	0.01

^{a,b,c} Within rows, values not followed by the same superscript differ significantly (P<0.05).

¹ Average of 7 chicks.

² Commercial diet.

The Fe-unsupplemented diet significantly decreased body weight already at 7 d of age (Table 33). Chicks fed the diets containing 50 and 150 mg Fe/kg had similar BWs, which were significantly lower than those of the chicks fed on the commercial diet since the age of 14 d. Dietary cholecalciferol level did not affect growth.

Blood hemoglobin concentrations at 27 d of age were significantly lower in chicks fed on the Fe-unsupplemented diet than in those fed the other diets (Table 34). Values observed in chicks fed the diets supplemented either with 50 or 150 mg Fe/kg and the commercial diet were essentially the same. Despite the significant difference in body weight between the chicks fed on the synthetic diet supplemented with 50 and 150 mg Fe/kg on the one hand, and those fed on the commercial diet on the other hand, absolute liver weights of these chicks were similar, and even at times significantly higher in chicks fed the diet supplemented with 150 mg Fe/kg. That, in turn, resulted in a significant difference in relative liver weight between the chicks fed on the synthetic diets and those fed on the commercial diet (Table 34). Birds fed on the Fe-unsupplemented diet had the highest relative liver weight.

The effect of the various treatments on a few variables involved in Ca metabolism are detailed in Table 35. Chicks fed the Fe-supplemented diets had significantly lower levels of calcium and higher levels of phosphorus in their plasma than those fed the Fe-unsupplemented diet, and the commercial diet. The concentrations of these minerals in the plasma of the birds fed on the last two diets were essentially the same. Chicks fed on the Fe-unsupplemented diet had the lowest concentration of duodenal calbindin. Fe supplementation significantly increased this variable to levels which were similar to that observed in birds fed on the commercial diet. Fe supplementation decreased the activity of kidney 25(OH)D₃-24-hydroxylase, and increased the activity of kidney 25(OH)D₃-1-hydroxylase, both effects were not significant. Chicks fed on the Fe-unsupplemented diet had the lowest concentration of ash in their tibiae. It is worth noting that increasing Fe supplementation from 50 to 150 mg/kg

tend to decrease, at times significantly, this variable. Neither of the variables was significantly affected by dietary Fe level, but the higher level tended to decrease ($P < 0.10$) the activity of kidney 25(OH)D₃-1-hydroxylase.

TABLE 33. Effect of Fe supplements to Fe-deficient diet and dietary cholecalciferol (CHOL) level on body weight during the course of Experiment 3^{1,2}

Dietary variables		Age (d)			
CHOL	Fe	7	14	21	27
(µg/kg)	(mg/kg)	Body wt (g)			
25	0	128 ^c	223 ^b	314 ^c	357 ^b
25	50	142 ^{abc}	306 ^a	551 ^b	816 ^a
25	150	146 ^{ab}	328 ^a	600 ^{ab}	895 ^a
62.5	0	137 ^{bc}	243 ^b	319 ^c	373 ^b
62.5	50	143 ^{ab}	318 ^a	576 ^{ab}	866 ^a
62.5	150	153 ^{ax}	342 ^{ay}	632 ^{ay}	924 ^{ay}
Commercial diet		154 ^x	382 ^x	708 ^x	1083 ^x
SEM		4.6	12.5	23.1	44.3
Factorial effects					
CHOL	25	139	286	488	689
CHOL	62.5	144	301	509	721
P<		NS	NS	NS	NS
Fe	0	132 ^b	233 ^b	316 ^b	365 ^b
Fe	50	142 ^a	312 ^a	563 ^a	841 ^a
Fe	150	149 ^a	335 ^a	618 ^a	910 ^a
P<		0.01	0.001	0.001	0.001
CHOL x Fe					
P<		NS	NS	NS	NS

^{a,b,c,x,y} Within columns, values of chicks not followed by the same superscript differ significantly ($P < 0.05$). The letters x and y are used to demonstrate differences between chicks fed on the commercial diet and those fed on the synthetic diets (see below).

¹ Average of 6 chicks. ANOVA was carried out twice, a factorial ANOVA with data of birds fed only the synthetic diets, and a simple ANOVA with data of all the treatments. The results of Duncan's test followed the latter are not detailed, except for the comparison between values observed in chicks fed on the commercial diet and the numerically closest value observed in the other treatments.

² Body wt at 1-d of age averaged 47 g.

TABLE 34. Effect of Fe supplements to Fe-deficient diet and dietary cholecalciferol (CHOL) level on body and liver weights, and the concentration of hemoglobin (Hem.) in blood of 27-d old broiler chickens (Experiment 3)¹

Dietary variables		Body	Liver	Liver	Blood
CHOL	Fe	wt	wt	wt	Hem.
(µg/kg)	(mg/kg)	(g)	(g)	(% BW)	(g/100 dl)
25	0	357 ^c	11.2 ^c	3.21 ^a	2.93 ^c
25	50	816 ^a	21.4 ^{bx}	2.61 ^c	9.43 ^{ax}
25	150	895 ^a	24.1 ^{ab}	2.70 ^{bc}	9.41 ^a
62.5	0	373 ^b	11.4 ^c	3.07 ^{ab}	3.15 ^c
62.5	50	866 ^a	21.9 ^b	2.53 ^{cx}	9.40 ^a
62.5	150	924 ^{ay}	26.4 ^a	2.86 ^{abc}	8.46 ^b
Commercial diet		1083 ^x	21.6 ^x	1.99 ^y	9.45 ^x
SEM		44.3	1.19	0.141	0.28
Factorial effects					
CHOL	25	689	18.9	2.84	7.26
CHOL	62.5	721	19.9	2.82	7.00
P<		NS	NS	NS	NS
Fe	0	365 ^b	11.3 ^c	3.14 ^a	3.04 ^b
Fe	50	841 ^a	21.6 ^b	2.57 ^b	9.41 ^a
Fe	150	909 ^a	25.3 ^a	2.78 ^b	8.93 ^a
P<		0.001	0.001	0.001	0.001
CHOL x Fe					
P<		NS	NS	NS	NS

^{a,b,c ,x,y} Within columns, values of chicks not followed by the same superscript differ significantly ($P < 0.05$). The letters x and y are used to demonstrate differences between chicks fed on the commercial diet and those fed on the synthetic diets (see below).

¹ Average of 6 chicks. ANOVA was carried out twice, a factorial ANOVA with data of birds fed only the synthetic diets, and a simple ANOVA with data of all the treatments. The results of Duncan's test followed the latter are not detailed, except for the comparison between values observed in chicks fed on the commercial diet and the numerically closest value observed in the other treatments.

TABLE 35. Effect of Fe supplements to Fe-deficient diet and dietary cholecalciferol (CHOL) level on plasma calcium and phosphorus levels, duodenal mucosa calbindin (calcium binding protein, CaBP) and the activities of 25(OH)D₃-hydroxylase (24-HYD) and 25(OH)D₃-1-hydroxylase (1-HYD) in the kidneys of 27-d old broiler chickens (Experiment 3)¹

Dietary variables		Blood		Duodenal	Kidney		Tibia ash
CHOL	Fe	Ca	P	CaBP	24-HYD	1-HYD	
(μ g/kg)	(mg/kg)	(g)	(g)	(μ g/g)	(pmole/g/15 min)		(%)
25	0	10.7 ^a	5.7 ^{bx}	315 ^b	14.7	69.7 ^b	27.3 ^c
25	50	8.4 ^c	7.1 ^{ab}	1128 ^a	11.1	119.6 ^{ab}	33.4 ^{ab}
25	150	7.9 ^b	8.4 ^a	1009 ^a	10.5	153.3 ^a	32.3 ^b
62.5	0	10.5 ^{ax}	5.5 ^b	280 ^b	15.1	94.1 ^{ab}	25.9 ^c
62.5	50	9.1 ^{ab}	7.8 ^a	1083 ^{ax}	10.8	71.5 ^b	34.9 ^{ax}
62.5	150	8.2 ^b	7.8 ^a	1315 ^a	12.8	71.9 ^b	33.5 ^b
Commercial diet		10.2 ^x	6.0 ^x	1155 ^x	ND ²	ND	36.8 ^x
SEM		0.55	0.55	153	1.71	21.9	0.88
Factorial effect							
CHOL	25	9.0	7.1	817	12.1	114.2	31.0
	62.5	9.3	7.0	929	12.9	79.2	31.4
P<		NS	NS	NS	NS	0.10	NS
Fe	0	10.6 ^a	5.6 ^b	298 ^b	14.9	81.9	26.6 ^b
	50	8.8 ^b	7.4 ^a	1104 ^a	11.6	95.5	34.2 ^a
	150	8.1 ^b	8.1 ^a	1177 ^a	10.9	112.6	32.9 ^a
P<		0.001	0.001	0.001	0.10	NS	0.001
CHOL x Fe							
P<		NS	NS	NS	NS	0.10	NS

a,b,c ,x,y Within columns, values of chicks not followed by the same superscript differ significantly (P<0.05). The letters x and y are used to demonstrate differences between chicks fed on the commercial diet and those fed on the synthetic diets (see below).

¹Average of 6 chicks. ANOVA was carried out twice, a factorial ANOVA with data of birds fed only the synthetic diets, and a simple ANOVA with data of all the treatments. The results of Duncan's test followed the latter are not detailed, except for the comparison between values observed in chicks fed on the commercial diet and the numerically closest value observed in the other treatments.

²Not determined.

Experiment 4 (chicks). The effect of the supplementation of the Fe-deficient diet containing 62.5 µg cholecalciferol/kg with four concentrations of Fe (0, 25, 50 and 150 mg/kg) on the development, the concentrations of hemoglobin, calcium and phosphorus in blood, tibia ash, duodenal calbindin and meat stability was determined. The experiment was terminated at 51 d of age.

The Fe-unsupplemented diet significantly decreased body weight already at 7 d of age (Table 36). Supplementation of this diet with 25 mg Fe/kg resulted in body weights which, up to 51 d of age, did not differ significantly from those observed in birds fed on the diet supplemented with 150 mg Fe/kg. There was no significant difference in body weights between birds of the last treatment and of those fed on the commercial diet. Body weights of birds fed on the diet containing 50 mg Fe/kg were lower since the age of 35 d than those of chicks fed on the diets supplemented with 25 and 150 mg Fe/kg, due to the presence of one chick whose growth was markedly impaired.

Blood hemoglobin concentrations at 51 d of age were significantly lower in chicks fed on the Fe-unsupplemented diet than in those fed on the Fe-supplemented diets were similar to those of birds fed on the other diets (Table 37). Absolute liver weights of birds fed on the Fe-supplemented diets were similar to those of the birds fed on the commercial diet, while the relative weight of the livers of the last mentioned treatment was significantly lower. Chicks fed the Fe-unsupplemented diet had the highest relative liver weight. Chicks fed on the Fe-unsupplemented diet had the highest calcium and the lowest phosphorus levels in the plasma, as well as the lowest concentration of duodenal calbindin (Table 38). Fe

supplementation significantly increased the last variable. Intermediate values were observed in birds fed on the commercial diet. Tibia ash concentration was the lowest in chicks fed on the Fe-unsupplemented diet and the highest in chicks fed the diet supplemented with 50 mg Fe/kg.

TABLE 36. Effect of Fe supplements to Fe-deficient diet on body weight (g) during the course of Experiment 4^{1,2}

Age (d)	Basal diet				CD ³	SEM	P<
	Fe-deficient						
	Fe supplementation (mg/kg)						
	0	25	50	150			
7	135 ^b	146 ^{ab}	151 ^a	152 ^a	149 ^a	4.4	0.05
14	236 ^c	318 ^b	330 ^b	344 ^{ab}	378 ^a	13.1	0.001
21	342 ^c	574 ^b	571 ^b	648 ^{ab}	719 ^a	29.5	0.001
27	411 ^c	824 ^b	804 ^b	970 ^{ab}	1061 ^a	55.5	0.001
35	518 ^d	1193 ^{bc}	1074 ^c	1417 ^{ab}	1573 ^a	97.6	0.001
42	641 ^d	1634 ^{bc}	1457 ^c	1941 ^{ab}	2079 ^a	123.8	0.001
49	782 ^d	2087 ^{bc}	1823 ^c	2383 ^{ab}	2594 ^a	145.6	0.001
51	839 ^d	2247 ^{bc}	1940 ^c	2518 ^{ab}	2748 ^a	151.6	0.001

a,b,c Within rows, values not followed by the same superscript differ significantly (P<0.05).

¹ Average of 6 chicks.

² Body wt. at 1 d of age average 47 g.

³ Commercial diet.

TABLE 37. Effect of Fe supplements (Fe sup.) to Fe-deficient diet on body and liver weights (g), and the concentration of hemoglobin in blood of 51-d old broiler chicks (Experiment 4)¹

Fe sup. (mg/kg)	Body wt. (g)	Liver wt (g)	Liver wt. (g)	Hemoglobin (g/100 ml)
0	839 ^d	24.8 ^b	2.94 ^a	3.5 ^c
25	2247 ^{bc}	51.1 ^a	2.30 ^b	8.1 ^b
50	1940 ^c	45.3 ^a	2.37 ^b	8.8 ^{ab}
150	2518 ^{ab}	52.9 ^a	2.11 ^b	9.2 ^a
Commercial diet	2748 ^a	45.9 ^a	1.68 ^c	9.0 ^{ab}
SEM	151.6	3.15	0.110	0.316
P<	0.001	0.001	0.001	0.001

a,b,c Within columns, values of chicks not followed by the same superscript differ significantly (P<0.05).

¹ Average of 6 chicks.

TABLE 38. Effect of Fe supplements to Fe-deficient diet on plasma calcium and phosphorus level, duodenal mucosa calbindin (calcium binding protein, CaBP) and tibia ash in 51-d old broiler chicks (Experiment 4)¹

Fe sup. (mg/kg)	Blood		Duodenal CaBP	Tibia ash
	Ca (mg/dl)	P (mg/dl)	(µg/g)	(%)
0	11.8 ^a	6.2 ^b	352 ^c	26.2 ^c
25	10.5 ^b	7.7 ^a	860 ^{ab}	31.1 ^b
50	9.9 ^b	6.9 ^{ab}	1039 ^a	36.6 ^a
150	9.9 ^b	7.4 ^a	790 ^{ab}	31.8 ^b
Commercial diet	10.6 ^b	6.6 ^{ab}	609 ^{bc}	32.7 ^b
SEM	0.25	0.37	87.0	1.04
P<	0.01	0.05	0.001	0.001

^{a,b,c}Within columns, values of chicks not followed by the same superscript differ significantly (P<0.05).

¹Average of 6 chicks.

Fe accumulation and α -tocopherol (chicks)

Data on the accumulation of total iron in liver and in muscle tissues from chicks on synthetic Fe-deficient diets and Fe-supplemented diets is summarized in Figures 18 and 19. The data demonstrated that a diet containing 10-20 mg Fe/kg resulted in a significantly lower amount of iron in liver and muscle tissues. Such an amount of iron in the diets also significantly decreases the concentration of blood hemoglobin (Tables 32, 34, 35, 37).

A significant increase in total tocopherol and α -tocopherol concentrations in the muscle was obtained with birds on an Fe-deficient diet, compared with birds on the high Fe-supplemented (150 mg/kg) diet (Figures 20, 21).

FIGS. 18. and 19. The effect of dietary Fe-supplementation level on total iron in chick liver and drumstick muscle (Experiments I, III, IV).

FIG. 20. The effect of dietary Fe-supplementation level on α -tocopherol in chick drumstick muscle (Experiments I, III, IV).

FIG. 21. The effect of dietary Fe-supplementation level on total tocopherols in chick muscle (Experiments I, III, IV).

The results demonstrated that *in vivo* iron plays a significant role on the metabolism of α -tocopherol. It is well known that Fe plays an important role in the catalysis of lipid peroxidation *in vivo* (Halliwell and Gutteridge, 1990).

It may be possible that tissues containing lower amounts of iron are on a lower rate of peroxidation, which decreases α -tocopherol oxidation, or its turnover.

Figure 22 shows that muscles from chicks fed a diet of high Fe-supplementation (150 mg/kg diet) have a higher rate of lipid peroxidation, compared with muscles from chicks fed a lower level of Fe-supplementation.

In contrast to the results of Experiment I, in which muscle lipid peroxidation was increased by a high level of Fe supplementation (Fig. 22); in Experiments III and IV, dietary Fe levels did not affect lipid peroxidation (data not presented). The phenomenon was probably due to the high level of α -tocopherol in the muscle of chicks from Experiments III and IV, compared with the level observed in Experiment I (Fig. 20).

Muscle stability from chicks on a commercial diet was found to be relatively very stable. This stability could be attributed to the high concentration of α -tocopherol (2.8 - 3.4 $\mu\text{g/g}$ wet wt.) and total tocopherols (6 $\mu\text{g/g}$ wet wt.) present in the muscle of chicks fed the commercial diet (results not presented).

FIG. 22. The effect of dietary Fe-supplementation (0, 25, 150 mg Fe/kg diet) on drumstick muscle lipid peroxidation stored at 4°C (Experiment 1).

Enzymatic activities of SOD, catalase and glutathione peroxidase in turkey muscle tissues

Measurement of defense oxidation enzymes in tissues homogenates was done by a method developed by Del Maestro and McDonald (1985).

The enzyme activities were determined in meat of turkeys raised on diets of low and high Fe (25 and 500 mg/kg diet) and low and high Vit. E (0, 28, 150 mg/kg diet) (Experiment 5).

Superoxide dismutase activities show that drumstick muscles contain between 150 and 250 units/g wet tissues and no significant differences

were obtained between the treatments and after storage of the muscle at 4°C for a period of 10 days (data not shown).

Catalase activities were high after slaughtering (15-25 units/g wet tissues) and decreased significantly after 8 days of storage (4-8 units). Samples from turkeys fed a high Vit. E diet, after slaughtering, showed a higher catalase activity than samples taken from turkeys fed a low Vit. E diet.

Very interesting data were obtained by determining the glutathione peroxidase activity of turkey muscle tissues.

Glutathione peroxidase in muscle tissues from all the experiments was relatively small (~1 unit/g wet tissue) a day after slaughtering and increased 5-8 fold after incubation of the muscle at 4°C for 4-8 days (Fig. 23).

This dramatic increase in glutathione peroxidase activity could be attributed to (a) *de novo* synthesis of the enzyme; (b) decreasing of an inhibitor; and (c) increasing the release of the enzyme from a bound form.

Increasing Vitamin E in the diet was found to decrease enzyme activities but only in samples fed a high Fe diet (Fig. 24).

This enzyme which is known to decompose hydrogen peroxide and lipid hydroperoxides to non-radical products, seems to have an important effect on the rate of lipid peroxidation in muscle food during storage.

Glutathione peroxidase was also determined in drumsticks (tissues showing a rapid lipid peroxidation during storage at 4°C) and breast (tissue with a low rate of lipid peroxidation). The data show a significant increase in the enzyme activity from drumsticks but not from breast tissue, during storage at 4°C (Fig. 25).

FIG. 23. Catalase activity in drumstick muscles of turkeys fed diets with various amounts of Vitamin E and Fe (mg/kg) stored at 4°C (Experiment 5).

Fe-20, Vit. E 0, ○; Fe-20, Vit. E 28, Δ; Fe-20, Vit. E 150, □; Fe-500, Vit. E 0, ○; Fe-500, Vit. E 28, Δ; Fe-500, Vit. E 150, □.

FIG. 24. Glutathione peroxidase activity in drumstick muscles of turkeys fed diets with various amounts of Vitamin E and Fe (mg/kg), as affected by storage at 4°C (Experiment 5).

FIG. 25. Glutathione peroxidase activity in drumstick and breast muscles of turkeys fed on a commercial diet, stored at 4°C. D=drumstick; B=breast.

The results of this study did not demonstrate a clear effect of dietary Fe level on meat stability of turkeys. High levels of Fe decreased the stability in one experiment (Experiment 3) and is in agreement with our previous report (Kanner *et al.*, 1990). However, in the other experiments, this effect was not evident. We have no explanation for this disagreement, which was probably due to an effect of ferrous ions on the stability of dietary lipid and vitamin E under certain conditions (Rao and Artz, 1989).

The results demonstrated that intake of excess iron by diet or by injections to turkeys, chicks or swine led to hepatic, and to a lesser degree, skeletal muscle iron accumulation. Iron accumulation in tissues led to a corresponding decrease in the concentration of vitamin E and in some experiments to increase in the susceptibility of the tissues to lipid peroxidation.

Our results, in part, support other reports which show that *in vivo* lipid peroxidation has been produced in human iron-overload (Rachmilewitz *et al.*, 1976; Heys and Dormondy, 1981; Halliwell and Gutteridge, 1990), as well as in experimental animals (Dougherty *et al.*, 1981; Dillard *et al.*, 1984; Marklund *et al.*, 1981; Nayini *et al.*, 1985). Most recently, it was reported that consuming dietary iron (3000, 5000 and 8000 mg/kg diet) compared with control diet (120 mg/kg diet) in mice resulted in accumulation of iron in the liver and depletion of vitamin E (Omara & Blakley, 1993).

These results and ours are both in agreement with an earlier report by Rachmilewitz *et al.* (1976) that in iron-loaded patients with thalassemia

major, excess lipid peroxidation in erythrocytes is associated with reduced serum vitamin E concentration.

We have demonstrated, in muscles of iron-deficient chicks, a higher concentration of α -tocopherol and less *in vivo* oxidation of this compound than in muscles from chicks fed on diets with normal or high iron levels. Muscles from iron-deficient chicks showed a decrease in lipid peroxidation during storage at 4°C.

Our results demonstrate new evidence that iron depletion in anemic chicks increases the concentration of α -tocopherol in tissues. These results demonstrate that iron, in general, plays an important role in the tone and rate of *in vivo* (or *ex vivo*) lipid peroxidation of tissues, and not only on those with iron-overload toxicity.

Iron depletion, defined as the absence of stored iron without anemia, was recommended most recently as strategic to increase the antioxidative tone in humans (Sullivan, 1994). The evidence for powerful antioxidant effects of iron depletion *in vivo* is growing (Salonen *et al.*, 1992).

In swine and especially in turkey and poultry diets, iron depletion is not practical as an Fe-unsupplemented commercial diet already contained a high concentration of iron which prevents the depletion of iron stores from the liver or muscle tissues. Increasing the amount of vitamin E in the diet should be the chosen treatment for decrease muscle food susceptibility to lipid peroxidation.

SUMMARY

Swine

- (1) Non-heme iron and TBARS were higher in cooked pork from pigs fed high iron diets.
- (2) Cooking increased TBARS but did not affect non-heme iron in Pork Skeletal Muscles.
- (3) Non-heme iron and TBARS increased continuously during storage while α -tocopherol decreased in skeletal muscle, but especially in ground pork.
- (4) The common practice of heavily fortifying swine rations with iron (≥ 200 ppm) increased non-heme iron and TBARS in cooked, stored meat, but did not contribute significantly to warmed-over aroma (WOA). TBARS were strongly correlated with WOA during storage and with non-heme iron.
- (5) Moderate elevation in iron stores, especially in liver, had a marked effect on oxidative stress, as indicated by the increase in TBARS in swine.
- (6) Supplementation with vitamin E, and to a lesser extent vitamin C, protected against this oxidative stress.
- (7) Cooking increased TBARS and caused a dramatic reduction in vitamin E levels in ground pork.

Turkey and chicks

- (1) Unsupplementation of Fe in practical diets of turkeys and chickens did not affect body weight or blood hemoglobin levels, because they contained "natural" ~120 mg Fe/kg feed.
- (2) High amounts of supplemental Fe (500 mg/kg diet) significantly increased blood hemoglobin only in one experiment, but did not affect body and liver weight, and only slightly affected muscle iron level.
- (3) Blood levels of hemoglobin and total iron in muscle of turkey, but not "free" iron, increased with age.
- (4) Most of the experiments involving turkeys (3 out of 4) did not show a significant effect of dietary Fe level on the stability of the muscle tissues to lipid peroxidation.
- (5) Injection of Fe-dextran to the left drumstick of turkeys significantly increased the total and "free" iron in this tissue and in the liver, but only slightly in the right drumstick.
- (6) Injection of Fe-dextran to the left drumstick of turkeys significantly decreased the amount of α -tocopherol in both drumsticks, and in the liver.

- (7) Injection of Fe-dextran to the left drumstick of turkeys significantly decreased the stability of the left drumstick and the thigh, determined by accumulation of TBARS. Dietary vitamin E supplementation (150 mg/kg diet) partly prevented this decrease. The effect of Fe-dextran injection on decreasing the stability of the right drumstick and thigh was negligible.
- (8) Lipid peroxidation in muscle turkey tissue stored at 4°C or -18°C was significantly inhibited by increasing the level of dietary vitamin E.
- (9) Iron loading decreased α -tocopherol levels in the muscles of turkeys, chickens and swine.
- (10) The effect of supplementation on an Fe-deficient synthetic diet with various concentrations of Fe (0, 25 and 150 mg/kg) on chick development, blood hemoglobin and meat stability was also evaluated. The chicks fed the Fe-deficient diet showed a decrease in body weight, blood hemoglobin, and a significant increase in 25 D₃-1 hydroxylase. The results demonstrate that the optimal level of Fe for growth, blood hemoglobin and the calcification of the tibia is ca. 50 mg/kg diet.
- (11) Increasing Fe-supplementation from 0 to 50 mg Fe/kg diet increased total iron in the liver and muscle tissue, and significantly decreased the accumulation of α -tocopherol in muscle tissues. That, in turn, affected, although not consistently, the stability of muscles. The results demonstrate that Fe played a special role in *in*

vivo lipid peroxidation, which affects the turnover and accumulation of α -tocopherol.

- (12) Several defense enzymes were determined and found in the turkey muscle, such as superoxide dismutase, catalase, and glutathione peroxidase. Glutathione peroxidase was more active in muscles with a high trend of lipid peroxidation (drumsticks vs. breast muscles, and muscles with a low α -tocopherol content). The activity of glutathione peroxidase increased several fold in muscle stored at 4°C.

CONCLUSIONS

- (1) The common practice of heavily fortifying swine rations with iron (> 200 ppm) increased non-heme iron and TBARS in cooked, stored meat, but did not contribute significantly to warmed-over aroma (WOA). TBARS were strongly related with WOA.
- (2) Diets of turkeys or chickens, based on common feedstuffs, not supplemented with Fe, did not reduce body weight, blood hemoglobin level, or iron pool in the liver or muscle. The reason being that these diets contained "natural" ~120 mg Fe/kg feed, and this amount was found to be high enough to keep constant the pool of iron in the body, liver or muscle tissues.
- (3) Only high levels of supplemental Fe (500 mg/kg) significantly increased, at times, blood hemoglobin and total iron in the liver of

turkeys, but these levels hardly affect iron pool in the muscles. It seems that the liver may accumulate very high concentrations of iron and regulates very efficiently iron concentration in skeletal muscles. For this reason, it was very difficult to decrease muscle stability in turkeys through a diet containing high levels of Fe-supplementation.

- (4) It was shown that the significant increase in the amount of iron (total and "free") in the muscle occurred after injections with Fe-dextran, accelerated lipid peroxidation and decreased the concentration of α -tocopherol in the muscles.
- (5) Treatments which could significantly decrease or increase iron pool and its metabolism in muscle tissues (or other organs) may affect the rate of lipid peroxidation and the turnover of α -tocopherol.
- (6) Our work demonstrated that it will be much more practical to increase the stability of muscle tissues in swine, turkeys and chickens during storage and processing by increasing the amount of vitamin E in the diet than by withdrawing iron supplementation.

BIBLIOGRAPHY

- Addis, P.B. and Warner, G.J. (1991). The potential health aspects of lipid oxidation products in food. In: (Aruoma O.C., Halliwell, B., eds.) *Free radicals and food additives*. London: Taylor and Francis Ltd., pp. 77.
- Aisen, P. and Liskowsky, I. (1980). Iron transport and storage proteins. *Ann. Rev. Biochem.* 49: 357.
- Ames, B.N. (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science* 221: 1256.
- Ang, C.Y.W. (1988) Comparison of broiler tissues for oxidative changes after cooking and refrigerated storage. *J. Food Sci.* 53: 1072.
- Anon (1984). Total hemoglobin: Quantitative colorimetric determination in whole blood at 530-550 nm. Procedure No. 525, Sigma Chemical Co., St. Louis, MO, U.S.A. p. 20.
- Anon (1988a). Prediction of rancidity essential when extending shelf-life of processed foods. Prepared Foods, February, Academy Press, Washington DC.
- Anon, N.R.C. (1988). Nutrient requirements of domestic animals. Nutrient requirements of swine. Ninth ed. National Research Council, Washington DC.
- Arnold, R.N., Arp, S.C., Scheller, K.K., Williams, S.N. and Shaefer, D.M. (1993). Tissue equilibration and subcellular distribution of vitamin E in displayed beef. *J. Anim. Sci.* 71: 105.
- Aust, D.S. (1988). In: Oxygen Radical and Tissue Injury, Proceedings of Brook Lodge Symposium, Michigan, U.S.A. (B. Halliwell, ed.,) pp. 27.

- Bar, A. and Hurwitz, S. (1979). The interaction between dietary calcium and gonadal hormones in their effect on plasma calcium, bone, 25-hydroxycholecalciferol-1-hydroxylase, and duodenal calcium-binding protein, measured by a radioimmunoassay in chicks. *Endocrinology* 104: 1455.
- Bar, A., Rosenberg, J., Perlman, R. and Hurwitz, S. (1987). Field rickets in turkeys: Relationship to vitamin D. *Poultry Sci.* 66: 68.
- Bartov, I. and Burnstein, S. (1977). Stability of abdominal fat and meat of broilers. Relative effects of vitamin E, BHT and ethoxyquin. *Br. Poult. Sci.* 18: 59.
- Beecher, G.R., Cassens, R.G., Hoekstra, W.G. and Briskey, E.J. (1965). Red and white fiber content and associated post-mortem properties of seven porcine muscles. *J. Food Sci.* 30: 969.
- Biemond, P., Swaak, A.J.G., Beindorff, C.M. and Koster, J.F. (1986). Superoxide-dependent and -independent mechanisms of iron mobilization from ferritin by xanthine oxidase. *Biochem. J.* 239: 169.
- Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911.
- Boyer, R.F. and McCleary, J.C. (1987). Superoxide ion as a primary reductant in ascorbic-mediated ferritin iron release. *J. Free Rad. Biol. & Med.* 3: 389.
- Buchowski, M.S., Mahoney, A.W., Carpenter, C.E. and Cornforth, D.P. (1988). Heating and the distribution of total and heme iron between meat and broth. *J. Food Sci.* 53: 43.
- Burton, G.W., Webb, A. and Ingold, K.U. (1985). A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. *Lipids* 20: 1.

- Chan, K.M. and Deker, E.A. (1994). Endogenous skeletal muscle antioxidants. *CRC Reviews in Fol. Sci. and Nutr.* 34: 403.
- Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H. and Ku, P.K. (1984). Some factors influencing non-heme iron content of meat and its implication in oxidation. *J. Food Sci.* 49: 581.
- Dabbagh, A.J., Morrison, T., Lynch, S.M. and Frei, B. (1994). The effect of iron overload on rat plasma and liver oxidant status *in vivo*. *Biochem. J.* 300: 799.
- Decker, E.A. and Hultin, H.O. (1990). Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci.* 55: 947, 953.
- Decker, E.A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 38: 674.
- Del Maestro, R.F. and McDonald, W. (1985). Oxidative enzymes in tissue homogenates. *CRC Handbook of Methods for Oxygen Radical Research*, ed. Robert A. Greenwald, CRC Press p. 291.
- Dillard, C.J., Downey, J.E. and Tappel, A.L. (1984). Effect of antioxidants of lipid peroxidation in iron-loaded rats. *Lipids* 19: 127.
- Dougherty, J.J., Croft, W.A. and Hoekstra, W.G. (1981). Effects of ferrous chloride and iron-dextran on lipid peroxidation *in vivo* in vitamin E and selenium-adequate and -deficient rats. *J. Nutr.* 111: 1784.
- Dugan, L.R. (1980). In *Autooxidation in Food and Biological Systems* (Simic, M.C. and Karel, M., eds.). Plenum Press, New York, p. 261.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*. 11: 1.
- Dupuy, H.P., Bailey, M.E., St. Angelo, A.J., Vercellotti, J.R. and Legendre, M.G. (1988). Instrumental Analyses of Volatiles Related to Warmed-Over Flavor of Cooked Meats. Ch. 7, In *Warmed-Over*

- Flavor of Meat*, A.J. St. Angelo and M.E. Bailey (eds.), p. 165, Academic Press, Inc., Orlando, FL.
- Ewan, R.C. (1985). Analysis of feeds and feed ingredients - 1984. ISU Research Reports - 1985. Iowa State Univ., Ames, IA.
- Ewan, R.C. (1986). Analysis of feeds and feed ingredients - 1985. ISU Research Reports - 1986. Iowa State Univ., Ames, IA.
- Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D. and Merkel, R.A. (eds.) (1975). Structure and Composition of Muscle and Associated Tissues. Ch. 3, In *Principles of Meat Science*, pp. 25-81. W.H. Freeman and Company, New York, NY.
- Frazzolari, F.A. (ed.) (1978). *Compilation of Odor and Taste Threshold Values Data*, American Society for Testing and Materials, Philadelphia, PA.
- Fridovich, I. (1983). Superoxide radical: an endogenous toxicant. *Ann. Rev. Pharmacol. Toxicol.* 23: 239.
- German, J.B. and Kinsella, J.E. (1985). Lipid oxidation in fish tissue enzymatic initiation via epoxygenase. *J. Agric. Food Chem.* 33: 680.
- Gower, J.D., Healing, G. and Green, C.D. (1989). Determination of desferrioxamin available iron in biological tissues by HPLC. *Anal. Biochem.* 180: 126-130.
- Graf, E. and Panter, S.S. (1991). Inhibition of warmed-over flavor development by polyvalent cations. *J. Food Sci.* 56: 1055, 1067.
- Grossman, S., Bergman, M. and Sklan, D. (1988). Lipoxygenase in chicken muscle. *J. Agric. Food Chem.* 36: 1268.
- Gutteridge, J.M.C. (1986). Iron promoters of the Fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides. *FEBS Lett.* 201: 291.

- Halliwell, B. and Gutteridge, J.M.C. (1985). The importance of free radicals and catalytic metal ions in human diseases. *Molec. Aspects Med.* 8: 89.
- Halliwell, B. and Gutteridge, J.M.C. (1986). Oxygen-free radicals and iron in relation to biology and medicine. *Archives of Biochem. Biophys.* 246: 501.
- Halliwell, B. and Gutteridge, J.M.C. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. In *Methods in Enzymology*, vol. 186, part B, 1, (Lester Packer and Alexander N. Glazer, eds.) Academic Press.
- Han, D., McMillin, K.W., Godber, J.S., Bidner, T.D., Younathan, M.T., Marshall, D.L. and Hart, L.T. (1993). Iron distribution in heated beef and chicken muscles. *J. Food Sci.* 58: 697.
- Hara, A. and Radin, N.S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* 90: 420.
- Harel, S. and Kanner, J. (1985). Hydrogen peroxide generation in ground muscle tissues. *J. Agric. Food Chem.* 33: 1186.
- Harel, S., Salan, M.D. and Kanner, J. (1988). Iron release from metmyoglobin, methemoglobin and cytochrome C by a system generating H₂O₂. *Free Rad. Res. Comms.* 5: 11.
- Harel, S. and Kanner, J. (1988). The generation of ferryl or hydroxyl radicals during interaction of haemproteins with hydrogen peroxide. *Free Rad. Res. Comms.* 5: 21.
- Harel, S. and Kanner, J. (1989). Hemoglobin and myoglobin as inhibitors of Hydroxyl Radicals Generation in a Model System of "Iron Redox" Cycle. *Free Rad. Res. Comms.* 6: 1.
- Hershko, C. and Weatherall, D.J. (1988). Iron-chelating therapy. *CRC Critical Reviews in Clinical Laboratory Sciences* 26: 4, 303.

- Heys, A.D. and Dormandy, T.L. (1981). Lipid peroxidation in iron-overloaded spleens. *Clin. Sci.* 60: 295.
- Hsieh, R.J. and Kinsella, J.E. (1989). Lipoxygenase generation of specific volatile flavor carbonil compounds in fish tissues. *J. Agric. Food Chem.* 37: 279.
- Iancu, T.C. (1982). Iron overload. *Molec. Aspects Med.* 6: 1.
- Igene, J.O. and Pearson, A.M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *J. Food Sci.* 44: 1258.
- Jacobs, A. (1977). Iron overload: Clinical and Pathological Aspects. *Semin. Haemt.* 14: 89.
- Kanner, J. (1974). Ph.D. Thesis, Univ. of Jerusalem, Jerusalem, Israel.
- Kanner, J., Harel, S., Shagalovich, J. and Berman, S. (1984). Antioxidative effect of nitrite in cured meat products. Nitric oxide-iron complex of low molecular weight. *J. Agric. Food Chem.* 32: 512.
- Kanner, J. and Harel, S. (1985a). Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. *Arch. Biochem. Biophys.* 237: 314.
- Kanner, J. and Harel, S. (1985b). Lipid peroxidation and oxidation of several compounds by activated metmyoglobin. *Lipids* 20: 625.
- Kanner, J., Harel, S. and Hazan, B. (1986). Muscle membranal lipid peroxidation by an "Iron Redox Cycle" system: Initiation by oxyradicals and site-specific mechanism. *J. Agric. Food Chem.* 34: 506.
- Kanner, J., German, J.B. and Kinsella, J.E. (1987). Initiation of lipid peroxidation in biological systems. *CRC Clinical Reviews in Food Sci. and Nutr.* 25: 317.

- Kanner, J. and Bartov, I. (1988). *Meshek Haofot* 24: 805 (in Hebrew).
- Kanner, J., Shagalovich, I., Harel, S. and Hazan, B. (1988a). Muscle lipid peroxidation dependent on oxygen and free metal ions. *J. Agric. Food Chem.* 36: 409.
- Kanner, J., Hazan, B. and Doll, L. (1988b). Catalytic "Free" Iron in muscle foods. *J. Agric. Food Chem.* 36: 412.
- Kanner, J., Sofer, F., Harel, S. and Doll, L. (1988c). Antioxidant activity of ceruloplasmin in muscle membranal and *in situ* lipid peroxidation. *J. Agric. Food Chem.* 36: 415.
- Kanner, J., Bartov, I., Salan, M.O. and Doll, L. (1990). Effect of dietary iron level on *in situ* turkey muscle lipid peroxidation. *J. Agric. Food Chem.* 38: 601.
- Kanner, J., Hazan, B. and Doll, L. (1991a). Catalytic "free" iron ions in muscle foods. *J. Agric. Food Chem.* 36: 412.
- Kanner, J., Salan, M.O., Harel, S. and Shagalovich, I. (1991b). Lipid peroxidation of muscle food: The role of the cytosolic fraction. *J. Agric. Food Chem.* 39: 242.
- Kanner, J., Harel, S. and Jaffe, R. (1991c). Lipid peroxidation of muscle food as affected by NaCl. *J. Agric. Food Chem.* 39: 1017.
- Kanner, J. and Doll, L. (1991). Ferritin in turkey muscle tissue: A source of catalytic iron ions for lipid peroxidation. *J. Agric. Food Chem.* 39: 247.
- Kanner, J. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* 36: 169.
- Khayat, A. and Schwall, D. (1983). Lipid oxidation in seafood. *Food Technol.* 37: 130.
- Lannek, N., Lindberg, P. and Tollerz, G. (1962). Lowered resistance to iron in vitamin E deficient piglets and mice. *Nature* 195: 1006.

- Lee, Y.H., Layman, D.K., Bell, R.R. and Norton, H.W. (1981). Response of glutathione peroxidase and catalase to excess dietary iron in rats. *J. Nutr.* 111: 2195.
- Marklund, S.L. (1985). Pyrogallol oxidation in CRC Handbook of Methods for Oxygen Radical Research, Robert A. Greenwald (ed.). CRC Press p. 291.
- McShane, L.M., Clark, L.C., Combs, G.F. and Turnbull, B.W. (1991). Reporting the accuracy of biochemical measurements for epidemiologic and nutrition studies. *Am. J. Clin. Nutr.* 53: 1.
- Melton, S.L. (1983). Methodology for following lipid oxidation in muscle foods. *Food Technol.* 37(7): 105, 116.
- Melton, S.L., Davidson, P.M. and Mount, J.R. (1987). Sensory Analysis of Undesirable Flavors in Meat. Ch. 6, In *Warmed-Over Flavor of Meat*, A.J. St. Angelo and M.E. Bailey (eds).
- Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* 38: 514.
- Miller, D.K. (1992). Unpublished data. Dept. of Food Science, Cornell Univ., Ithaca, NY.
- Miller, D.K., Gomez-Basauri, J.V., Smith, V.L. and Miller, D.D. (1994a). Dietary iron concentration in swine rations affects non-heme iron and thiobarbituric acid reactive substances in pork skeletal muscles. *J. Food Sci.* 59: 747.
- Miller, D.K., Smith, V.L., Kanner, J., Miller, D.D. and Lawless, H.T. (1994b). Lipid oxidation and warmed-over aroma (WOA) in cooked ground pork from swine fed varying levels of iron. *J. Food Sci.* 59: 750.

- Morrison, D.R. and Campbell, A.M. (1971). Phospholipids as related to total lipid and DNA in light and dark portions of porcine semitendinosus muscle. *J. Food Sci.* 36: 1102-1103.
- Murray, M.J., Murray, A.B. and Murray, N.J. (1991). Do iron and copper supplementation of the diet impair antioxidant activity and speed atherogenesis? Arteriosclerosis Council Abstracts. *Arterioscler. and Thromb.* 11: 1479a.
- National Research Council (1984). Nutrient Requirements of Poultry, 8th rev. ed. National Academy Press, Washington DC.
- National Research Council (1988). National Academy Press, Washington DC. p. 15.
- Nayini, N.R., White, B.C., Aust, S.D., Huang, R.R., Fudriere, R.J., Evans, A.T., Bialek, H., Jacobs, W.A. and Komora, J. (1985). Post-resuscitation iron delocalization and malondialdehyde production in brain following prolonged cardiac arrest. *J. Free Radic. Biol. Med.* 1: 111.
- Omara, F.O. and Blakley, B.R. (1993). Vitamin E is protective against iron toxicity and iron-induced hepatic vitamin E deficiency in mice. *J. Nutr.* 123: 1649.
- Rachmilewitz, E.A., Lubin, B.H. and Shohet, S.B. (1976). Lipid membrane peroxidation in β -thalassemia major. *Blood* 47: 495.
- Pearson, A.M. and Tauber, F.W. (1984). Raw Materials, Ch. 6, In *Processed Meats*. Second ed., p. 106. Avi Publishing Company, Inc., Westport, CT.
- Pikul, J., Leszczynski, D.E. and Kummerow, F.A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* 37: 1309.

- Pond, W.G., Yen, J.T. and Crouse, J.D. (1989). Tissue mineral element content in swine fed clinoptilolite. *Bull. Environ. Contam. Toxicol.* 42: 735.
- Poste, L.M., Willemot, C., Butler, G. and Patterson, C. (1986). Sensory aroma scores and TBA values as indices of warmed-over flavor in pork. *J. Food Sci.* 51: 886.
- Rao, S.K. and Artz, (1989). Effect of extension on lipid peroxidation. *J. Food Sci.* 54: 1580.
- Rhee, K.S., Ziprin, Y.A. and Ordonez, G. (1987). Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-H₂O₂, non-heme iron and enzyme systems. *J. Agric. Food Chem.* 35: 1013.
- Rhee, K.S. and Ziprin, Y.A. (1987). Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. *J. Food Sci.* 52: 1174.
- Salonen, J.T., Salonen, R., Nyyssonen, K. and Korpela, H. (1992). Iron sufficiency is associated with hypertension and excess risk of myocardial infarction. *The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). Circulation* 85: 864.
- Sarris, V. and Parducci, A. (1978). Multiple anchoring of category rating scales. *Perception and Psychophysics* 24: 35.
- Schricker, B.R., Miller, D.D. and Stouffer, J.R. (1982). Measurement and content of nonheme and total iron in muscle. *J. Food Sci.* 47: 740.
- Schricker, B.R. and Miller, D.D. (1983). Effects of cooking and chemical treatment on heme and nonheme iron in meat. *J. Food Sci.* 48: 1340, 1349.
- Seman, D.L., Decker, E.A. and Crum, A.D. (1991). Factors affecting catalysis of lipid oxidation by a ferritin-containing extract of beef muscle. *J. Food Sci.* 56: 356.

- Simic, M.G. and Karel, M. (1980). Autoxidation in Food and Biological Systems. Plenum Press, NY.
- Snedecor, G.D. and Cochran, W.G. (1967). Statistical Methods, 6th ed. Iowa State Univ. Press, Ames, IA.
- St. Angelo, A.J. and Bailey, M.E. (1987). Warmed-over Flavor of Meat. Academic Press, Orlando, FL.
- Steinberg, D., Parthasarathy, S., Carew, T.E. and Witztum, J.L. (1989). Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *New England J. Med.* 320: 915.
- Stevens, J.C. (1971). Psychophysical Problems and Procedures, Ch. 1, In *Stimulus and Sensation*, W.S. Cain and L.E. Marks (eds.), p. 5.
- Stewart, J.C.M. (1980). Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Biochem.* 104: 10.
- Stookey, L.L. (1970). Ferrozine - a new spectrophotometric reagent for iron. *Anal. Chem.* 42: 779.
- Sullivan, J.L. (1988). Iron, plasma antioxidants, and the oxygen radical disease of prematurity. *Am. J. of Diseases of Children* 142: 1341.
- Sullivan, J.L. (1994). Antioxidant effects of iron depletion. *Free Rad. Biol. & Med.* 17: 87.
- Tarladgis, B.G., Watts, B.M., M.T. and Dugan, L.R. (1960). A distillation method for the quantitative determination of malondialdehyde in rancid foods. *J. Am. Oil Chem. Soc.* 37: 44-48.
- Tellefson, C.S., Bowers, J.A., Marshall, C. and Dayton, A.D. (1982). Aroma, color, and lipid oxidation of turkey muscle emulsions. *J. Food Sci.* 47: 393.
- Theil, C. Elizabeth and Aisen, P. (1987). The storage and transport of iron in animal cells. In *Iron Transport in Microbes, Plants and*

Animals. G. Winkelman, D. Van der Helm and J.B. Neilands (eds.).
WCH FRG, pp. 491.

Willemot, C., Poste, L.M., Salvador, J., Wood, D.F. and Butler, G. (1985).
Lipid degradation in pork during warmed-over flavour development.
Can. Inst. Food Sci. Technol. J. 18(4): 316.

Wilson, B.R., Pearson, A.M. and Shorland, F.B. (1976). Effect of total
lipids and phospholipids on warmer-over flavor in red and white
muscles from several species as measured by TBA analysis. *J.*
Agric. Food Chem. 24: 7.

Witte, V.C., Krause, G.F. and Bailey, M.E. (1970). A new extraction
method for determining 2-thiobarbituric acid values of pork and beef
during storage. *J. Food Sci.* 35: 582.

Yamauchi, K., Nagai, Y. and Ohashi, T. (1980). Quantitative relationship
between alpha-tocopherol and polyunsaturated fatty acids and its
connection to development of oxidative rancidity in porcine skeletal
muscle. *Agric. Biol. Chem.* 44: 1061.

DESCRIPTION OF COOPERATION

Owing to Prof. Kinsella's move from Cornell to Davis University, all the research in the U.S.A. was undertaken by Prof. Miller, who was one of the proposers of the original research, and was responsible for the research in the U.S.A.

Over four years, the joint Israeli and the American research groups were in close contact, exchanged reports and consulted each other about the joint research planning.

The Israeli team had the full cooperation of Dr. Ido Bartov who was responsible for nutritional research, and Prof. Kanner who was the head researcher and responsible for the research from a technological and biochemical viewpoint. Prof. Kanner visited the U.S.A. prior to the commencement of the research to coordinate the team cooperation.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

- (1) The conditions influencing the content of the iron levels in animal muscle were studied using nutritional means. In practice, it is difficult to reduce the iron level in the feed to levels which will decrease the iron level in muscle and will affect the lipid oxidation rate simultaneously, when the feed is based on common feedstuffs. This mainly stems from the presence of a high level of iron in the raw feed materials, even without iron supplementation.

Experiments with synthetic feed (whose iron content could be controlled to achieve very low iron levels) showed that iron levels below approx. 20 mg/kg of feed significantly inhibited chicken development. Iron ion levels below 20 mg/kg significantly reduce the muscle iron and lipid peroxidation levels.

- (2) Low, medium and high iron levels were given to animals and poultry over various experimental periods. It was found that at very low levels, under 20 mg/kg, and at high levels over 500 mg/kg, it was possible to affect the muscle iron levels. It was also possible to obtain significant changes in the muscle iron levels, when iron ions were injected. It was found that the liver regulates the muscle iron levels and most of the iron accumulates there.
- (3) Follow up of the muscle and liver iron levels after slaughter in swine, turkeys and chickens was carried out.

- (4) The overall relationship between iron in the feed and the level of “free” iron and antioxidative systems were studied throughout the research. It was found that it is possible to influence the level of the “free” iron only under extreme nutritional conditions, either very low or extremely high dietary Fe levels. An interesting and important finding was revealed in original experiments on low iron levels in poultry. An inverse proportion between iron in the feed and the membrane vitamin E levels was found. With very low levels of iron, there was a significant increase in the membrane α -tocopherol level. This finding suggests that the oxidation level and the vitamin exchange in the tissue had started at a base peroxidation level that is influenced by the muscle iron level. Apparently basic peroxidation, occurring in all tissues, is influenced by the iron ions and corrected by vitamin E.

It was found that tissue subjected to high lipid oxidation encouraged the production of glutathione peroxidase, which is the primary enzyme that breaks down hydroperoxides in tissue.

- (5) Muscle tissue oxidation was examined at a temperature of 4°C and after storage at -18°C, as required by the original program.

This research has expanded our knowledge of lipid oxidation in muscle products and its influence on quality. The research demonstrated that it is possible to control the peroxidation process by administering vitamin E at a level that will ensure the prevention of peroxidation. In turkey meat, the recommended vitamin level is that which causes the accumulation of about 10 mg α -tocopherol per gram of fresh muscle.

Increasing meat stability by omitting the iron supplement from commercial poultry or swine feed is not practical since diets based on common feedstuffs contained relatively high levels of Fe.

Our findings will help in the future design of more suitable nutrition for poultry and swine to achieve better nutritional quality meat.

LIST OF PUBLICATIONS

- Miller, D.K., Gomez-Basauri, J.V., Smith, V.L., Kanner, J. and Miller, D.D. (1994). Dietary iron concentration in swine rations affects non-heme iron and thiobarbituric acid reactive substances in pork skeletal muscle. *J. Food Sci.* 59: 747-750.
- Miller, D.K., Smith, V.L., Kanner, J., Miller, D.D. and Lawless, H.T. (1994). Lipid oxidation and warmed-over aroma (WOA) in cooked ground pork from swine fed varying levels of iron. *J. Food Sci.* 59: 750-756.
- Kanner, J., Harel, S., Hanzon, I. and Bartov, I. (1995). Decreasing nutritional iron in the chick diet increased muscle membranous α -tocopherol accumulation. *J. Agr. Food Chem.* (in preparation).
- Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Sci.* 36: 169-189.
- Kanner, J. (1992). Mechanism of non-enzymatic lipid peroxidation in muscle foods. *Amer. Chem. Soc. Series* pp. 55-73.
- Bartov, I. and Kanner, J. (1996). Effect of high levels of dietary iron and vitamin E on the stability of turkey meat (in preparation).